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APPARATUS AND METHOD FOR MEASURING CARDIAC VAGAL TONE

The present invention relates to a method and apparatus for measuring vagal tone, a neurophysiological signal generated within the brain and transmitted to the heart for controlling heart rate. The present invention also relates to a method of and apparatus for diagnosing medical conditions and, particularly but not exclusively, autonomic dysfunction conditions such as is found in spongiform encephalopathies.

The two neural mechanisms for controlling heart rate in the human and animal are the sympathetic and parasympathetic nervous systems. Sympathetic activity gives rise to relatively slowly varying changes in heart rate (e.g. below 0.1Hz). Parasympathetic activity is generated in a region of the brain known as the Vital Centre, which is located in the lower medulla, and is transmitted to receptors in the sino-atrial node of the heart along the vagus nerve. The vagus nerve is myelinated such that parasympathetic activity is conveyed rapidly to the heart. The continuous flow of signal conveyed along the vagus nerve is termed the 'vagal tone'.

Vagal tone tends to act as a 'brake' on the heart, slowing the heart rate to a lesser or greater extent. A high level of vagal tone also tends to give rise to relatively large and rapid fluctuations in heart rate period. Conventionally, it is these fluctuations which are used to measure vagal tone from recorded electrocardiograms (ECG) and to 'isolate' vagal tone from the relatively slowly varying effects of sympathetic activity. More particularly, vagal tone is generally measured by considering an ECG over a relatively long time period (e.g. 1000 beats) and evaluating the mean of the differences between consecutive beats (see for example Heart Rate variability, Eds. Merck Malik and A. John Comm. 1995, Futura Publishing).



It is believed that certain diseases and conditions (e.g. diabetes and respiratory tract obstructions) can adversely effect cardiac function via parasympathetic activity. There has long been a desire therefore for a method for accurately measuring vagal tone for the purpose of monitoring, and possibly diagnosing, such diseases and conditions, a desire which is not fully satisfied by conventional methods of measuring vagal tone. An accurate measure of vagal tone could also be useful in monitoring the activity of pharmaceuticals which are intended to effect parasympathetic activity or which have this effect as a side effect.

It is an object of the present invention to provide an apparatus and method for measuring vagal tone in real time from ECG recordings taken from a human or animal patient which obviates or mitigates at least one disadvantage of existing apparatus and methods.

According to a first aspect of the present invention there is provided apparatus for providing a measure of vagal tone in a human or animal patient and comprising:

- a) input means for receiving an ECG signal obtained from a patient;
- b) detection means coupled to said input for detecting QRS waveforms in said ECG signal and for generating timing data indicative of the periods between consecutively received QRS waveforms; and
- c) frequency demodulation means coupled to said detection means for demodulating said timing data substantially in real time and for generating a signal corresponding to the frequency modulating signal in said ECG signal, wherein said generated signal is used to provide said measure of vagal tone.

The present invention has resulted from the realisation that heart rate variability results in the frequency modulation of what is effectively a constant frequency nominal heart rate. This is analogous to frequency modulation of for example radio signals where

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an underlying carrier signal of constant frequency has its instantaneous frequency modulated by an information or modulating signal.

Preferably, the detection means is arranged to
5 compare received portions of the ECG signal against a stored expected QRS waveform (or portion thereof), to identify when a QRS complex is received. Preferably, said timing signal comprises a sequence of fixed length pulses which are generated upon receipt of a QRS
10 waveform. The spacing between the pulses of the sequence therefore corresponds to the spacing between the received QRS waveforms.

The frequency demodulation means of the present invention may be a simple frequency discriminator, for
15 example comprising a differentiator in series with an envelope detector. Alternatively, the frequency demodulation means may comprise a phase locked loop (PLL) incorporating a voltage controlled oscillator (VCO) substantially tuned to the nominal constant heart rate.
20 This nominal rate or frequency of the VCO may be preset or may be determined by sweeping the VCO frequency across a suitable range until a lock is obtained with the nominal heart rate frequency of the received ECG signal. A possible problem with these types of FM demodulators
25 however is that they may not function adequately where large variations of the nominal heart rate occur. For example, it is possible for the nominal heart rate in a human to vary between 30 and 200 beats per minute (bpm), with the vagal tone causing high frequency modulation of
30 the nominal rate.

A preferred form of demodulation means therefore comprises an integrator coupled to the detection means for receiving said fixed length pulses and a high pass filter and a low pass filter coupled separately to the
35 integrator for receiving the output of the integrator. The output of the high pass filter is coupled to a first voltage controlled oscillator via a second integrator



whilst the output of the low pass filter is coupled to a second voltage controlled oscillator. The outputs of the two voltage controlled oscillators are in turn coupled to respective inputs of a phase comparator arranged to generate at its output a signal indicative of the phase difference between the two input signals.

The component parts of the FM demodulator are selected so that for a constant, or slowly varying nominal heart rate, the outputs of the two voltage controlled oscillators are substantially in phase. In particular, the first mentioned integrator may have a time constant of between 1.0 and 2.5 seconds, e.g. 2 seconds. Preferably, the second mentioned integrator also has a time constant selected from this range.

The output from the phase comparator is preferably coupled to a third integrator, which again may have a time constant selected from the range 1.0 to 2.5 seconds.

Preferably, a pre-set DC bias is added to the output of the third integrator to ensure that the resulting summed signal always exceeds zero.

Preferably, the apparatus of the present invention comprises a filter coupled to the frequency demodulation means (or incorporated into that means) for filtering the frequency modulating signal to remove noise and other unwanted signal components. In particular, the filter may be arranged to remove signal components which arise from sympathetic control of the heart rate. As discussed above, sympathetic activity gives rise to relatively low frequency variations in heart rate and the filter may therefore comprise a low pass and/or high pass filter for allowing removal of low frequency sympathetic components of the modulation signal.

The apparatus preferably comprises signal processing means for converting the modulating signal into a linear vagal scale (LVS).

The apparatus of the present invention may be implemented in hardware or in software. Alternatively,

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the apparatus may be implemented by way of combination of hardware and software components.

In one embodiment, the apparatus is provided as a hand-held unit which has a display for displaying measured vagal tone.

According to a second aspect of the present invention there is provided apparatus for measuring vagal tone from an ECG according to the above first aspect of the present invention in combination with means for recording the ECG, coupled to said input.

The recording means may comprise for example electrodes for attachment to the patient and amplifiers for pre-amplifying the recorded ECG.

According to third aspect of the present invention there is provided a method for providing a measure of vagal tone from an ECG recorded from a human or animal patient, the method comprising the steps of:

detecting the occurrence of QRS waveforms in the ECG signal;

generating a timing signal indicative of the time period between consecutively received QRS waveforms;

frequency demodulating said timing signal in real time to obtain a frequency modulation signal, wherein said modulation signal is used to provide said measure of vagal tone.

Preferably, said generating step comprises generating a constant length pulse whenever a QRS waveform is detected.

Preferably, the method comprises the further step of filtering the ECG, the timing signal, or the modulation signal to remove low frequency components (e.g. due to sympathetic activity).

Preferably, the frequency demodulating step comprises integrating the generated pulse sequence, and separately high pass and low pass filtering the integrated signal. The high pass filtered signal is then integrated and the resulting signal used to frequency



modulate a carrier signal. The low pass filtered signal is separately used to frequency modulate a further carrier signal and the phase differences between the two modulated carrier signals determined.

5 According to a fourth aspect of the present invention there is provided apparatus for use in diagnosing spongiform encephalopathies in a human or animal patient, the apparatus comprising the apparatus of the above first or second aspect of the present
10 invention.

Preferably, the said apparatus is apparatus for use in diagnosing bovine spongiform encephalopathy.

According to a fifth aspect of the present invention there is provided a method of facilitating diagnosis of
15 bovine spongiform (BSE) encephalopathy in cattle, the method comprising;

measuring the vagal tone of a subject cow using the method of the above third aspect of the present invention;

20 comparing the measured vagal tone against the vagal tone expected for a healthy cow; and

providing an indication of the difference between the two compared tones to assist in the diagnosing of BSE.

25 The method may also comprise the step of diagnosing BSE if said difference exceeds a predefined threshold level.

For a better understanding of the present invention and in order to show how the same may be carried into effect reference will now be made, by way of example, to
30 the accompanying drawings in which:

Figure 1 is a pictorial view of a human and a cow showing the approximate positions of electrodes for recording an ECG;

35 Figure 2 is a block diagram of apparatus for measuring vagal tone from an incoming ECG;

Figure 3 shows in more detail a block diagram of one



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possible FM demodulator for the apparatus of Figure 2;

Figure 4 is a schematic block diagram of an alternative implementation of the apparatus of Figure 2;

Figure 5A-H illustrates the waveforms present at various points in the apparatus of Figure 4 for a constant heart rate of 30 beats per minute;

Figure 6 illustrates the waveform present at the output of an integrator of the apparatus of Figure 4 for a constant heart rate of 200 beats per minute;

Figure 7 shows the recorded resting heart rate in beats per minute (bpm) and simultaneous measured vagal tone (CIPA - Cardiac index of parasympathetic activity) over 350 consecutive cardiac cycles for a healthy Fresian heifer aged 2.5 years; and

Figure 8 shows data identical to that shown in Figure 7 but with a compressed vertical scale;

Figure 9 shows the data of Figure 7 over 250 consecutive cardiac cycles for a healthy adult Fresian cow;

Figure 10 shows data identical to that shown in Figure 9 but with a compressed vertical scale; and

Figure 11 shows the data of Figure 7 for a six year old Fresian cross Aberdeen Angus cow diagnosed (post-mortem) with BSE.

Reference is first made to Figures 1 and 2 in which Figure 2 shows the apparatus for measuring vagal tone from an ECG signal recorded using electrodes positioned as shown in Figure 1. The ECG signal is supplied to the input 1 of a QRS waveform detector 2. The detector 2 provides an output in the form of a train of pulses of constant width and 1 volt amplitude, a new pulse being generated each time a QRS waveform is detected. The spacing between the pulses therefore corresponds to the spacing between consecutive QRS waveforms.

In its simplest embodiment, the detector may comprise a threshold detector which examines the ECG signal for waveforms which exceed a predefined threshold.

However, such a detection method may generate pulses whenever a noise spike of sufficient amplitude is present in the ECG signals. As such spikes are common, the simple threshold detection method is generally unsuitable. A preferred detection method involves comparing the shape of received waveforms against a stored waveform, which stored waveform is typical of QRS waveforms. Providing that the degree of similarity is adequate, a received waveform is identified as a QRS waveform and a pulse generated at the output of the detector 2.

The output of the detector is provided to the input of frequency modulation (FM) demodulator 3 and the output of the demodulator is, in turn, passed to a filter (integrator) 4 and the output of this is passed to a vagal tone gauge 5 for providing an output of vagal tone as will be more fully described with reference to Figure 4. Figure 3 illustrates one possible implementation of the FM demodulator 3. In particular, Figure 3 shows a phase locked loop in which the pulse train provided by the detector 2 is applied to one input 6a of two inputs 6a, 6b of a phase comparator 6. The output 6c of the phase comparator 6 is passed through a loop filter 7 to the output 7a of the FM demodulator 3. The output 7a of the FM demodulator 3 is fed back to a voltage controlled oscillator (VCO) 8 which generates a pulse train having a frequency dependent upon the applied input voltage. The pulse train output by the VCO 8 is applied to the second input 6b of the phase comparator 6. The VCO 8 is arranged to operate at a nominal frequency (i.e. when the input to the VCO is zero volts) equal to the nominal heart rate frequency. The phase comparator 6 detects any difference in the frequencies of the pulse trains provided by the VCO 8 and the QRS waveform detector 2 and, via the feedback loop, tends to drive the VCO 8 so as to eliminate the difference. The output 7a is therefore indicative of the frequency modulation of the

ECG.

In order to set the nominal frequency of the VCO 8 to the nominal heart rate, means are provided for superimposing a ramp input voltage to the VCO 8 to sweep the nominal frequency of the across an appropriate range. Once frequency lock is achieved, the ramp may be disconnected.

There is illustrated in Figure 4 an alternative implementation of the apparatus of Figure 2 which is designed to operate across a wide range of nominal heart rates (30 to 200bpm) which may occur in a human with or without vagal tone. The apparatus comprises an input 9 for receiving an incoming ECG signal and for conveying the ECG signal to a QRS waveform detector 10. As already described, the detector 10 generates at its output a pulse each time a QRS waveform is detected in the input signal. The pulse train generated by the detector 10 is applied to the input of an integrator 11 which comprises a diode rectifier followed by a low pass filter (a parallel connected capacitor). The time constant of the integrator 11 is two seconds. The integrator 11 provides an output signal which has an amplitude related to the frequency of the pulse train input signal. The output 11a of the integrator is provided to both a high pass filter 12 and a low pass filter 13. The high pass filter 12 has a 3dB cut off frequency of 0.2Hz whilst the low pass filter 13 has a 3dB cut off frequency of 0.015Hz.

The output of the high pass filter 12 is provided to a second integrator 14 which is identical to the first mentioned integrator 11. This second integrator 14 provides a substantially DC output signal generally proportional to the amplitude of the AC signal passed by the high pass filter 12. The output of the second integrator 14 is in turn connected to the input of a VCO 15 which is a pulse generator arranged to generate constant length pulses at a frequency dependent upon the amplitude of the input voltage. This VCO 15 has a linear

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positive slope frequency/voltage response, an input voltage of 0 providing a frequency of 3Hz and an input voltage of 0.6 volts providing a frequency of 20Hz.

5 The output of the low pass filter 13 is similarly provided to a VCO 16 (there being no need to pass this signal through an integrator as the output of the low pass filter 13 is substantially DC or only slowly varying). This VCO 16 has a linear negative slope frequency voltage response, a voltage of 0.7 volts
10 providing a frequency of 20Hz and a voltage of 1 volt providing a frequency of 3Hz.

The two outputs from the VCOs 15,16 are provided to a phase detector 17 which, in turn, generates a further pulse train of constant duration pulses, where the
15 periods between the pulses are proportional to the phase differences between the pulse trains provided to the inputs of the phase detector 17. The output of the phase detector is applied to a third integrator 18 which generates an output signal having an amplitude
20 proportional to the frequency of the input pulses.

The output of the integrator is applied to a summation unit 19 where it is added to a constant DC level 19a. The output 19b of the summation unit is, in turn, applied to a vagal tone gauge 20 which applies a
25 multiplication factor to the summed signal. This factor is selected to convert the output into a standard linear vagal tone scale (see 'A Linear Scale for Measuring Vagal Tone in Man', J. Auton. Pharmacol (1992) 12,109-115). Typically the vagal tone gauge is implemented in
30 software, for example using a PC connected to receive the output of the summation unit 19.

The operation of the apparatus of Figure 4 will now be described with reference to Figure 5A-H which shows the waveforms present at various points A to H in the
35 apparatus for the case where an ECG (waveform 5A) is received which has a constant (nominal) rate of 30 beats per minute (the lowest expected human rate) and which is

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unmodulated, that is no vagal tone. Ideally, the output of the QRS waveform detector (Figure 5B) is a sequence of pulses which are spaced apart by two seconds and, as already stated above, the amplitude of these pulses is 1 volt. As the time constant of the integrator 11 is two seconds, the output of the integrator 11 (Figure 5C) will decay from a maximum amplitude of 1 volt to a minimum amplitude of around 0.37 volts before each new pulse is received. The output of the integrator 11 will therefore result in an AC component D, Figure 5, at the output of the high pass filter 12 of around 0.6 volts and a DC component F, Figure 5, at the output of the low pass filter 13 of around 0.7 volts. Whilst the DC output of the low pass filter 13 can be supplied directly to the input of the associated VCO, the output of the high pass filter 12 must be passed through the second integrator in order to provide a DC voltage E, Figure 5, (indicative of the AC voltage level) to drive the associated VCO 15. Given the VCO characteristics described above, both voltage controlled oscillators will output a pulse train G,H (Figure 5) having a frequency of 20Hz such that the output of the phase detector 17 will be substantially zero. The DC constant added by the summation unit 19 is set so that the output 11a of the unit is zero for this constant heart rate input, i.e. to remove any DC offset.

Figure 6 illustrates the waveform which will be present at the output of the integrator 11 when a constant unmodulated heart rate of 200 beats per minute (the maximum expected human heart rate) is provided to the input of apparatus. The output from the QRS waveform detector 10 will be a sequence of pulses having a period of approximately 0.3 seconds. The output C, Figure 6, of the integrator 11 decays only marginally between pulses. This results in the integrator 11 providing an output with a relatively high DC component, approaching 1 volt, and a very low amplitude AC component, approaching zero volts as seen in Figure 6. Both VCOs will therefore

generate pulse trains having frequencies of approximately 3Hz given the characteristics described above and again the output from the final integrator will be 0 indicating that no vagal tone is present.

5 As a result of the linear response of the VCOs, the output of the final integrator 18 will always be zero where the heart rate is constant or only slowly varying. However, where the ECG comprises relatively high frequency variations, the output of the integrator 14 in
10 the high frequency leg will vary in contrast to the input to the VCO 16 in the low frequency leg which will remain substantially constant (in the short term). The outputs of the two VCOs therefore differ in frequency and a time varying output results from the final integrator
15 18 indicating that a vagal tone is present.

Figures 7 and 8 shows the vagal tone (CIPA) measured for a healthy 2.5 year old Fresian heifer. Also shown is the recorded heart rate in bpm. The heart rate varies between 55 and 77 bpm whilst the vagal tone (CIPA) varies
20 between 0 and 5. Figures 9 and 10 show the same data for a healthy adult Fresian cow for which the heart rate varies between 60 and 66 bpm. Vagal tone does not exceed 6.

Figure 11 shows heart rate and vagal tone for a
25 suckler cow which was killed after the recordings were made. This cow was subsequently certified by the Ministry of Agriculture, Food and Fisheries as having BSE after post-mortem examination. The cow is bradycardic, having a heart rate below 45 bpm (and frequently
30 considerably slower). Vagal tone varies from 45 to 180 prior to the intravenous injection of atropine (as indicated by the arrow in Figure 11). Atropine is a parasympatholytic drug which blocks the parasympathetic nervous system. It can be seen that the result of
35 injecting atropine is that the vagal tone drops nearly to zero (0.4) while the heart rate recovers to around 81 bpm. The vagal tone measured for the BSE diagnosed cow

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is dramatically higher, and suffers greater variations, than that for the healthy cows.

It will be appreciated from the results of Figures 7 to 11 that vagal tone as measured by the apparatus described above provides a method of diagnosing BSE in cattle. Given that other autonomic dysfunctions produce a similar dysfunction of the parasympathetic nervous system or vagal tone and may also be used to help diagnose other such similar conditions, for example scrapie in sheep and CJD in humans.

The apparatus, system and method hereinbefore described has a variety of clinical applications as detailed below in the various publications and research studies attached as Appendices and incorporated herein by reference.

"Hypoglycaemia reduces cardiac vagal tone in human diabetic and control subjects" by G.A. Thomson, P.J. Galloway, P.O.O. Julu, S. Hansen, G.A. Jamal, B.M. Fisher, C.G. Semple. (Appendix 1 pages 15 to 29).

"Sleep deprivation and its effect on an index of cardiac parasympathetic activity in early Non-REM sleep in normal and epileptic subjects" by R. Shane Delamont, Peter O.O. Julu and Goran A. Jamal. (Appendix 2 pages 30 to 50).

"Periodicity of a non-invasive measure of cardiac vagal tone during non-REM sleep in non-sleep deprived and sleep deprived normal subjects" by R. Shane Delamont, Peter O.O. Julu and Goran A. Jamal. (Appendix 3 pages 51 to 75).

"Immaturity of medullary cardiorespiratory neurones leading to inappropriate autonomic reactions is a likely cause of sudden death in Rett syndrome" by P.O.O. Julu, A.M. Kerr, S. Hansen, F. Apartopoulos, G.A. Jamal. (Appendix 4 page 76).

"Continuous measurement of the cardiac component of arterial baroreflex (CCBR) in real time during isometric exercise in human volunteers" by Peter O.O. Julu, S. Hansen, A. Barnes and G.A. Jamal. (Appendix 5 page 77).

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selective and dependant index of cardiac tone. "Real-Time Measurement of Cardiac Vagal Tone in Conscious Dogs" by Little C.J.L., Juluo P.O.O., Hansen S., Reid S.W.J. (Appendix 6 pages 78 to 94).

- 5 Also clinical applications are disclosed in a) European Child & Adolescent Psychiatry 6:1-8 (1997) Suppl. 1 entitled "Functional evidence of brain stem immaturity in Rett syndrome"; (Reference 1 pages 95 to 102) and b) Neurology 49, December 1997 entitled
- 10 "Selective defect of baroreflex blood pressure buffering with intact cardioinhibition in a woman with familial aniridia; (Reference 2 pages 103 to 105).

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Hypoglycaemia reduces cardiac vagal tone in human diabetic and control subjects.

APPENDIX 1

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Short Title: Cardiac vagal tone during hypoglycaemia

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Abstract

Cardiac vagal tone and heart rate were investigated during hypoglycaemia in six patients with type 1 diabetes and six control subjects.

Resting cardiac vagal tone was greater in diabetic patients than control subjects ($p < 0.05$). During hypoglycaemia cardiac vagal tone fell from 5.6 (4.3-8.6) to 2.1 (1.3-4.1) linear vagal scale (LVS) units in control subjects ($p < 0.05$) and 11.3 (8.1-14.8) to 3.4 (1.9-4.6) LVS units in diabetic patients ($p < 0.05$). Cardiac vagal tone then increased to 6.0 (5.1-11.8) and 16.1 (6.2-25.8) LVS units ($p = \text{NS}$ in both cases) respectively.

Basal heart rates were not significantly different. During hypoglycaemia heart rate increased from 70 (63-77) to 90 (78-99) beats min^{-1} in control subjects ($p < 0.05$), and 63 (57-67) to 88 (78-102) beats min^{-1} in diabetic patients ($p < 0.05$). Heart rates subsequently returned to basal levels in both groups.

Our findings suggest that changes in cardiac parasympathetic tone may underlie the heart rate effects of hypoglycaemia.

Key Words:

Parasympathetic, autonomic nervous system, cardiac vagal tone, hypoglycaemia, type 1 diabetes, insulin.

Introduction

Hypoglycaemia stimulates a heterogeneous autonomic response^(1, 2). Parasympathetic fibres travel mainly with the vagus nerve, and lie in close association to the deep viscera. Direct neurophysiological recordings in humans have not been possible for this reason. Plasma catecholamine concentrations and somatic nerve sympathetic activity can be measured directly, and most studies of autonomic activity during hypoglycaemia have therefore concentrated upon these responses⁽³⁻⁶⁾. Hypoglycaemia induces a prolonged increase in sympathetic activity^(7, 8), with a β_1 receptor mediated⁽⁹⁾ increase in heart rate. It has been suggested that cardiac vagal tone increases to oppose the sympathetic effect on heart rate^(9,10).

Cardiac vagal impulses can now be quantified non-invasively. These impulses are integrated in the sino-atrial node, and tend to affect heart periods beat-by-beat⁽¹¹⁾. They are largely initiated by baroreceptor stimulation⁽¹¹⁾ and are virtually withdrawn when this is removed⁽¹²⁾. Baroreceptor stimulation causes delayed onset of the subsequent cardiac cycle, and this is reflected in electrocardiogram (ECG) intervals⁽¹³⁾. Repeated stimulation of the baroreceptors during each cardiac cycle causes quantifiable pulse synchronised fast alterations in the ECG R-R intervals. A continuous assessment of cardiac vagal tone can be obtained from these, and quantified using a linear vagal scale (LVS) derived using atropine⁽¹⁴⁾.

The present study was performed to assess the effects of acute hypoglycaemia on cardiac vagal tone and heart rate in control subjects and diabetic patients. Using atropine in control subjects, a separate assessment of the validity of the cardiac vagal tone measurements was also made.

Subjects

Six male patients with type 1 diabetes (median age 36.5 years, range 28-58 years; median duration of diabetes 15 years, range 10-17 years) were matched with six non-diabetic male control subjects (median age 31.5, range 24-39 years). All patients had well controlled diabetes (median haemoglobin A_{1c} 7%, range 6-8.3%; laboratory reference range 4.6-6.2%). None of the diabetic patients had a history of antecedent hypoglycaemic episodes in the six weeks prior to the study, recurrent hypoglycaemia or hypoglycaemia unawareness. There was no evidence of diabetic complications in any of the diabetic patients, as assessed by fundoscopy, normal microalbumin excretion, and the absence of neuropathy on clinical examination including supine and erect blood pressure measurement. Approval was received from the local ethical committee and informed consent was obtained from all subjects.

Subjects were non-smokers with no history of ischaemic heart disease or epilepsy. To achieve euglycaemia prior to the study in diabetic subjects, evening isophane insulin was omitted and dextrose and insulin¹² (Human Actrapid, Novo Nordisk Pharmaceuticals Ltd., Crawley, West Sussex, UK.) were infused overnight. Subjects were fasted overnight and an indwelling antecubital venous cannula was inserted 30 minutes prior to commencing the study. The cannula was flushed with 2 mls of heparinised 0.9% saline (heparin, 1 unit/ml) between samples. Two mls of blood was discarded before each sample was drawn.

Induction of hypoglycaemia and glucose analysis

Acute hypoglycaemia was induced in all subjects with an intravenous bolus of insulin (Human Actrapid, 0.15 units/Kg). All subjects remained supine throughout the study. Plasma glucose was estimated basally (B), at the time of the maximal autonomic reaction to hypoglycaemia (R), and at $R + 30$ minutes, $R - 60$ minutes, $R + 120$ minutes and $R + 240$ minutes. The autonomic reaction to hypoglycaemia was determined by the onset the typical autonomic features¹²: tachycardia, widening of the pulse pressure, tremor, sweating and palpitations. Glucose analysis was performed using a standard automated glucose oxidase method (Beckman Synchron CX5, and Beckman glucose reagent kit; Beckman Instruments (UK) Ltd., High Wycombe, UK.).

Measurement of cardiac vagal tone

Cardiac vagal tone (CVT) was measured continuously from B-10 minutes till R + 240 minutes. Consecutive R-R intervals were obtained from an analogue ECG using a Neuroscope (Pontoppidan, Copenhagen, Denmark). Briefly the neuroscope acts as an interface between an analogue ECG machine connected to the subject and an IBM compatible microcomputer. By sampling the ECG signal at a frequency of 5 KHz, individual R-R intervals are extracted by the neuroscope and communicated to the microcomputer. Using a time domain analysis model, instantaneous analysis of these R-R intervals by the microcomputer (Vagosoft analysis software, Department of Neurophysiology, University of Glasgow, Glasgow, UK) provides a continuous real-time record of cardiac vagal tone. Cardiac vagal tone was measured in units according to a linear vagal scale (LVS units) which has previously been derived from experiments using atropine to abolish cardiac vagal tone¹⁴⁾. A continuous record of heart rate was also made from the ECG.

Validation of cardiac vagal tone measurements

A separate group of six healthy male volunteers (median age 32 years, range 29-36 years) participated in a study to validate measurements of cardiac vagal tone derived from the neuroscope. Following antecubital venous cannulation, atropine sulphate (non-proprietary; atropine sulphate b.p. 600 mcg ml⁻¹) was administered in cumulative doses up to 2 mg to each subject who remained supine throughout the study. Dose-dependent cardiac vagal tone responses were measured using the neuroscope.

Statistical analysis

Non-parametric statistical analysis was performed on a microcomputer using Minitab statistical software (Minitab data analysis software - release 7.2 standard version, Minitab Inc., 3081 Enterprise Drive, State College, PA 16801, USA.). Changes within a group were compared using sign tests. Comparisons between groups were performed using Mann-Whitney tests for individual measures, and Kruskal-Wallis tests for repeated measures. Results are expressed as medians with the corresponding interquartile range, and n=6 in all cases. P values of less than 0.05 were considered significant. Results obtained in validation of cardiac vagal tone measurements were compared with paired t-tests using the same statistical software.

Results

Plasma glucose (Figure 1 and table 1)

Hypoglycaemia, judged by the onset of typical autonomic features⁽¹⁶⁾, occurred 28 (25.3-30.7) minutes and 33 (28.2-38.4) minutes following the administration of insulin in control subjects and diabetic patients respectively. Basal median plasma glucose concentrations of 5.1 (4.8-5.5) mmol l⁻¹ and 6.6 (3.8-8.0) mmol l⁻¹ fell significantly to nadirs at R of 1.4 (1.2-1.5) mmol l⁻¹ and 1.5 (1.1-1.7) mmol l⁻¹ in control subjects ($p<0.05$) and diabetic patients ($p<0.05$) respectively. No significant differences in plasma glucose were observed between the groups basally or at R. The median plasma glucose concentration was significantly lower at R + 120 minutes ($p<0.05$) and significantly higher at R + 240 minutes ($p<0.05$) in diabetic patients, but there was no significant difference on analysis of repeated measures.

Cardiac vagal tone during hypoglycaemia (Figure 1 and table 1)

Basal median CVT of 11.3 (8.1-14.8) LVS units was significantly higher in diabetic patients ($p<0.05$) than 5.6 (4.3-8.6) LVS units in control subjects. A significant fall in median cardiac vagal tone accompanied the autonomic reaction to hypoglycaemia in control subjects ($p<0.05$) and diabetic patients ($p<0.05$). Median cardiac vagal tone increased thereafter, and remained higher in diabetic patients than control subjects (Kruskal-Wallis test; $p<0.01$) throughout the study.

Relative heart rate during hypoglycaemia (Fig. 1 and table 1)

Basal heart rates of 70.1 (63-77) beats min⁻¹ and 63 (57-67) beats min⁻¹ were not significantly different in control subjects and diabetic patients respectively. A significant increase in median heart rate occurred during the autonomic reaction to hypoglycaemia in control subjects ($p<0.05$) and diabetic patients ($p<0.05$). Thereafter, median heart rates did not differ significantly from baseline in either group. Median heart rate was significantly lower in diabetic patients throughout the study (Kruskal-Wallis test; $p<0.01$).

Validation of cardiac vagal tone measurements (Table 2)

Administration of cumulative doses of atropine abolished cardiac vagal tone at a total dose of 2 mg. This change was significant ($p<0.001$; paired t-test) when compared with basal cardiac vagal tone.

Discussion

Evaluation of cardiac vagal tone can be performed in a number of ways and measurement of heart rate variability by power spectral analysis is often employed⁽¹⁷⁾. This technique is both time consuming and subject to a number of other criticisms^(17, 18). High frequency heart rate variability responses derived by spectral analysis are regarded as being of parasympathetic origin because they are abolished by atropine⁽¹⁷⁾. In the present study, atropine caused a similar effect using the neuroscope to measure cardiac vagal tone. This finding confirms that the neuroscope offers a satisfactory alternative to spectral analysis for the derivation of cardiac vagal tone.

The present study has demonstrated a significant reduction in cardiac vagal tone coincident with the autonomic reaction to hypoglycaemia. This response was similar in both groups of subjects, and may act in synergy with sympathetic activation which occurs at this time^(1, 3-4). In non-diabetic subjects hypoglycaemia stimulates vagally mediated secretion of saliva⁽¹⁹⁾ and pancreatic polypeptide⁽²⁾. However, our present findings suggest that cardiac vagal activity was inhibited, which might indicate that the various divisions of the parasympathetic nervous system respond to hypoglycaemia in different ways. These non-cardiac vagal responses are attenuated during hypoglycaemia in diabetic patients⁽²⁰⁾, however the present study has suggested that the cardiac vagal responses were not. We did not examine other measures of vagal afferent activity however, and it remains unclear whether these may have been attenuated, leaving the cardiac vagal reflex intact.

In the present study, increased heart rate accompanied the fall in cardiac vagal tone during hypoglycaemia, which coincides with elevation of serum catecholamines⁽²¹⁾ and sympathetic afferent activity^(2, 4, 22). It has previously been suggested that cardiac vagal tone increases in response to this sympathetic activation^(9, 10). The present study has demonstrated an initial fall in cardiac vagal tone, followed by recovery to normal or suprabasal values which coincided with the return of heart rate to basal values. This suggests that a component of the initial rapid increase in heart rate which occurred during hypoglycaemia may have been mediated by the reduction in cardiac vagal tone. It also confirms the increased cardiac vagal tone which has previously been observed^(9, 10).

Hyperinsulinaemia causes cardiac vagal withdrawal, however this effect is probably more modest than the changes presently observed during hypoglycaemia⁽²³⁾. Such changes reflect altered baroreceptor stimulation^(12, 13), thus in addition to the hyperinsulinaemia immediately following insulin administration, the blood pressure⁽²⁴⁾ or plasma volume⁽²⁵⁾ responses to hypoglycaemia may have been partly responsible.

Autonomic neuropathy was not formally excluded in diabetic patients during the present study, but was unlikely in the absence of other complications. Furthermore, this omission might have limited impact on the present observations because baroreflex and cardiac vagal changes may be dissociated from other aspects of autonomic dysfunction⁽²⁶⁾. Throughout the present study, cardiac vagal tone was increased and heart rate reduced in diabetic patients compared with control subjects. Due to its cardiac vagolytic effect⁽²³⁾, it is unlikely that insulin infusion was responsible for this effect in diabetic patients. Subjects were not matched for physical fitness or bed rest prior to the study, and these factors might explain the underlying differences.

Attenuated cardiac vagal tone accompanies heart failure and is associated with sudden death⁽²⁷⁻³⁰⁾. In addition studies in patients with heart disease have shown an increased risk of both atrial⁽³¹⁾ and ventricular⁽³²⁾ arrhythmias in patients with reduced cardiac vagal tone. During hypoglycaemia significant reductions in serum potassium concentration occur⁽³³⁾, and in diabetic patients there may also be silent myocardial ischaemia⁽³⁴⁾. The association of these factors with attenuated cardiac vagal tone during hypoglycaemia might potentiate the risk of arrhythmias, and may suggest a mechanism for hypoglycaemia associated sudden death.

The present study has demonstrated a significant fall in cardiac vagal tone during acute hypoglycaemia which might cause altered sympathovagal balance. Thereafter cardiac vagal tone increased in both control subjects and diabetic patients. We conclude that further evaluation of baroreflex and cardiac vagal responses to various plasma concentrations of glucose and insulin is necessary.

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Legend for table 1

Changes in plasma glucose, cardiac vagal tone and heart rate during hypoglycaemia. Reaction denotes the autonomic reaction. Results are expressed as median (interquartile range) and significant differences noted (Mann-Whitney test). Significant changes from baseline within groups (sign test) are denoted * = $p < 0.05$. In diabetic patients, cardiac vagal tone was significantly increased ($p < 0.01$) and heart rate significantly reduced ($p < 0.01$) throughout the study (Kruskal-Wallis test).

Legend for table 2

Changes in cardiac vagal tone in response to administration of intravenous atropine. Administration of atropine caused a significant fall in cardiac vagal tone ($p < 0.001$).

Legend for figure 1

Changes in plasma glucose, cardiac vagal tone (CVT) and heart rate (median $\pm$ quartile deviation) during acute hypoglycaemia, expressed as percentages relative to baseline in non-diabetic control subjects (solid squares) and in patients with type 1 diabetes (open squares). R denotes the autonomic reaction. No significant differences were noted between control subjects and diabetic.

Significant falls in plasma glucose and CVT and a significant increase in heart rate occurred at R in control subjects [$p < 0.05$ in all cases] and diabetic patients [$p < 0.05$ in all cases].

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Atropine Dose (mg)	Cardiac vagal tone (LVS units)	SEM
0	10.0	2.0
0.1	13.9	3.6
0.2	8.2	2.2
0.4	4.8	1.7
0.6	1.6	0.6
1	0.4	0.3
1.5	0.05	0.05
2	0	0

Table 2

Table 1

Time	Plasma Glucose			Cardiac Vagal Tone			Heart Rate		
	Control subjects	Diabetic patients	Mann-Whitney test	Control subjects	Diabetic patients	Mann-Whitney test	Control subjects	Diabetic patients	Mann-Whitney test
Basal	5.2 (4.8-5.5)	6.6 (3.8-8.0)	NS	5.6 (4.3-8.6)	11.3 (8.1-14.8)	p<0.05	70 (63-77)	63 (57-67)	NS
Reaction	1.4 (1.2-1.5) *	1.5 (1.1-1.7) *	NS	2.1 (1.3-4.1) *	3.4 (1.9-4.6) *	NS	90 (78-100) *	88 (78-102) *	NS
R + 30	2.9 (2.7-3.5) *	2.4 (1.8-2.7) *	NS	4.4 (3.5-11.4) NS	6.2 (2.2-9.5) NS	NS	74 (58-80) NS	71 (66-98) NS	NS
R + 60	3.5 (2.9-4.4) *	2.8 (1.4-3.1) *	NS	5.2 (3.9-12.6) NS	7.4 (6.2-25.9) NS	NS	67 (55-78) NS	70 (51-75) NS	NS
R + 120	4.9 (4.7-5.0) NS	4.1 (3.9-4.6) NS	NS	4.6 (4.3-13.4) NS	16.1 (9.5-22.1) NS	NS	69 (53-76) NS	57 (45-63) NS	NS
R + 240	5.1 (4.9-5.3) NS	8.7 (6.0-9.7) NS	NS	6.0 (5.1-11.8) NS	14.8 (10.2-18.7) NS	NS	72 (54-75) NS	51 (44-65) NS	NS

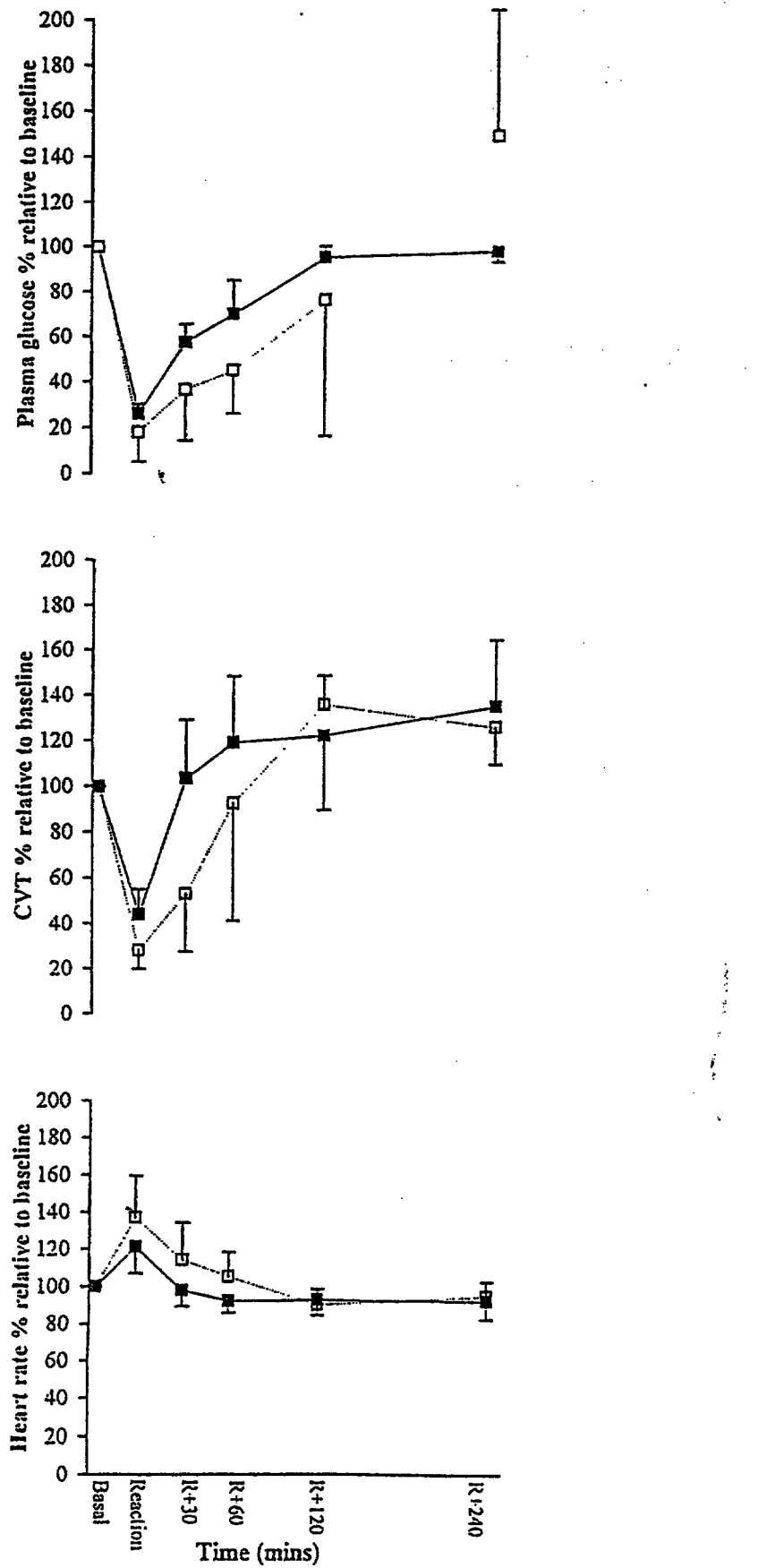


Figure 1

**Sleep deprivation and its effect on an index of cardiac
parasympathetic activity in early Non-REM sleep in normal and
epileptic subjects**

APPENDIX 2

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Running Title: Vagal tone in sleep deprivation

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Summary:

Using a recently developed real time measure of cardiac vagal tone calibrated against progressive doses of atropine, we examined the effect of 24 hour sleep deprivation on cardiac vagal tone during the first ultradian cycle of sleep in 16 subjects. The level of cardiac vagal tone was more than doubled in subjects who were sleep deprived compared to the level in non-sleep deprived subjects during sleep stage 0 immediately prior to the onset of sleep stage 1. The mean cardiac vagal tone fell significantly ($p < 0.01$) on reaching slow wave sleep stage 4 in sleep deprived epileptic subjects, and a similar trend in our sleep deprived normal subjects. There was a strong correlation between sleep stage and CIPA. We propose that the cardiac vagal tone may act as a marker of the homeostatic process S proposed by Borbély in his two process model of sleep. However, we were unable to demonstrate a fall in cardiac vagal tone during slow wave sleep stage 4 reported in non-sleep deprived subjects, possibly because our study was limited to the initial 90 minutes of sleep.

1. Introduction

Previous studies of the autonomic effects on the heart in sleeping humans have relied on indirect measures such as heart rate changes seen in different sleep stages throughout the night (1,2,3). Heart rate has been shown to decrease with an increase in respiratory sinus arrhythmia as sleep progressed from stage 1 to stage 4 and by using atropine and propranolol this heart rate change has been shown to be caused by augmented parasympathetic input. More recently heart rate variability has also been studied in different subjects including term infants (4), adults with diabetes mellitus (5), Alzheimer's disease (6), Parkinson's disease (7) and obstructive sleep apnoea (8) with similar findings though with evidence that autonomic control could be influenced by these disease processes in sleep. Spectral analysis of heart periods has provided some evidence of an increased parasympathetic activity in stage 2 sleep and a general reduction in autonomic neural activity including that of the parasympathetic system during slow wave sleep (SWS)(9). This is in contrast to earlier work suggesting an increase in parasympathetic activity in non REM (NREM) sleep particularly as SWS was reached (10,11). This discrepancy in the levels of parasympathetic activity in SWS, all of which were assessed respiratory sinus arrhythmia in heart periods, could be related to the starting levels of parasympathetic activity. When parasympathetic activity was high initially, it fell during SWS, but when the level was low initially, parasympathetic activity increased during SWS (10).

Although the recently developed technique of quantitative spectral analysis of heart rate variability is claimed to measure both sympathetic and parasympathetic influences on the heart non-invasively and without disturbing the patient in sleep (12), it probably does not measure these autonomic influences specifically (13). Moreover, this method cannot be time locked with the EEG in real-time and requires the kind of off-line analyses which are more suitable for research than routine clinical use.

A recently developed technique for assessing cardiac parasympathetic activity in real-time is now available (14). This new method uses a beat by beat index of cardiac parasympathetic activity (CIPA) which can be time locked with the EEG and is capable

of monitoring cardiac parasympathetic activity continuously. We validated this new method using atropine and subsequently applied it to study the effects of sleep deprivation on cardiac parasympathetic activity in the first ultradian cycle of sleep in a group of clinic patients and healthy volunteers using a clinical procedure for the investigation of epilepsy (15) routinely used in our department.

METHODS

Subjects

A total of twenty-one subjects were examined. Subjects in group N were used to validate the NeuroScope technique of measuring CIPA. The group consisted of five normal healthy volunteers aged between 24-40 years (mean age 32 years) receiving no drugs including alcohol. They were given cumulative doses of atropine up to 2.0 mg. intravenously within four hours while they were awake between 12 midday and 2100 hours and being monitored continuously at rest only, lying quietly in supine positions with their eyes open. Dose dependent response of CIPA to cumulative doses of atropine was determined in these subjects.

Group A consisted of seven normal and healthy volunteers who were not sleep deprived, 3 males aged between 28-45 years mean age (means $\pm$ SEM) of 36.3 ± 7 years and 4 females aged between 22-35 years with mean age of 30.2 ± 7 years. All these subjects were studied had no history of neurological illnesses and none was on drug treatment including alcohol. These subjects were studied from 1800 hours to midnight using standard sleep EEG montage: C3-A2, C4-A1; eye movement channels, chin muscle tone and continuous ECG recording. The data were stored in digital form on VHS video tapes using a Telefactor Beekeeper video telemetry system (Telefactor Corporation, Pennsylvania, USA) and subsequently converted back to analogue signal for transcription onto paper to enable sleep stage classification. The analogue ECG signal was fed into the

NeuroScope (Pontoppidan, Copenhagen, Denmark) for concurrent measurement of CIPA and heart rate (HR).

Group B consisted of five neurologically normal but sleep deprived subjects. None of these five subjects was on any drug treatment including alcohol at the time of the study.

Group C consisted of four sleep deprived epileptic subjects with a diagnosis of focal non-progressive epilepsy based on history, clinical, EEG and imaging records. All of these subjects were on anti-convulsant drug treatment at the time of the study.

The nine sleep deprived subjects in groups B and C together consisted of 5 males aged between 10-55 years with mean age of 32.8 ± 7 years; 4 females aged between 22-53 years, mean age of 36.5 ± 7 years. They were all studied using a standard protocol which entailed sleep deprivation for 24 hours prior to the recording of EEG and ECG in the afternoon between 1500 and 1800 hours. A 21 channel EEG and one ECG channel were recorded simultaneously for 90 minutes. Standard Ag/AgCl disc electrodes were placed on the scalp using the international 10:20 reference system and the EEG was recorded by a Nihon Kodan 4418 machine (Nihon Kodan, Tokyo, Japan) whilst analogue ECG signal from the patient was fed into the NeuroScope for concurrent measurement of CIPA and HR.

The records for all 16 subjects in groups A, B and C were divided sequentially into epochs 300 cardiac cycles long, determined from the ECG record to time-lock the EEG and ECG records and to optimize the calculation of mean values of CIPA during this period. These epochs were then staged using the Rechtschaffen and Kales¹⁶ guidelines. Only those epochs displaying a constant NREM sleep stage without intra-epoch sleep stage changes were used. REM sleep was excluded from this study. The awake EEG (stage 0) was taken as that epoch (300 cardiac cycles long) of quiet wakefulness immediately prior to the onset of sleep stage 1.

Measurement of cardiac index of parasympathetic (vagal) activity (CIPA)

The analogue ECG output from the video telemetry machine, or the Nihon Koden 4418 EEG machine was fed into the NeuroScope (Pontoppidan, Copenhagen, Denmark) for measurement of the beat-to-beat interval between consecutive ECG R-waves (R-R intervals) and CIPA. The NeuroScope measures consecutive heart periods as R-R intervals of ECG signals sampled at 5 kHz, and extracts the vagal effects on heart periods using an internal template similar to that published by Katona *et al.* (17). The CIPA measured by the NeuroScope was then expressed in units of a linear vagal scale (LVS) using the principles reported by Julu (14).

Heart rate (HR) was also calculated by the NeuroScope from R-R intervals. The NeuroScope was interfaced to a PC-microcomputer to facilitate on-line and simultaneous observation of the HR and CIPA in real time. Data were collected from each subject throughout the period of study and stored on hard disk for later retrieval and analysis.

Statistics

The values of CIPA and HR in different epochs were given as means together with their 95% confidence intervals. These means were compared using one-way ANOVA. Correlation analyses were performed, in which NREM sleep stages were used as ranks of the depth of sleep and the group means values of CIPA in the respective sleep stages within one group of subjects were regarded as normally distributed. We are able to say this for two reasons. The frequency distribution of CIPA is Gaussian in a healthy population (18) and secondly, the distribution of class means is normal no matter what the original population distribution was (19). The statistical operation above allows the correlation coefficient (r) from the regression of the parametric mean values of CIPA against the respective non-parametric sleep stages to be used for the test of association

between CIPA and the stages of sleep (20). Regression line was fitted using the least square method. Statistical significance was accepted at the 95% confidence level ($p < 0.05$). A commercial package STATGRAPHICS, version 5 (Statistical Graphics Corporation, Rockville, USA) was used for the statistical analyses.

RESULTS

Validation of the NeuroScope technique

The response of CIPA to atropine, measured in units of the LVS using the NeuroScope, in the subjects of group N is shown in Figure 1. A small dose of atropine increased the CVT from 10 to 14 units in the LVS, but subsequent cumulative doses decreased it in hyperbolic fashion (See Figure 1). The normal resting supine CVT in young adults is 10 units in the LVS (14). The CVT fell to 1.4 units in the LVS after 0.6 mg of atropine and it dropped further to zero after 1.5 mg. There was no further change in CVT following the administration of more atropine after reaching zero units at 1.5 mg (See Figure 1).

Figure 1 to be around here

General observations

Twenty-nine percent of the non-sleep deprived subjects in group A reached SWS within the first seven epochs after closing their eyes, whilst 67% of the sleep deprived subjects in groups B and C achieved SWS within this period. Overall, any subject with initial baseline values of CIPA greater than 14.0 units in the LVS in sleep stage 0 achieved SWS within 7 epochs of first entering stage 1 sleep, whereas those with baseline values of CIPA less than 14 units failed to achieve SWS within this period.

Effects of sleep deprivation on the baseline CIPA and heart rate in stage 0 sleep

The mean values of CIPA in group A during sleep stage 0 was 7.47 units in the LVS with 95% confidence limits between 5.21-9.72 units; mean HR was 77.3 beats/min. with the confidence limits between 72.1- 82.4 beats/min. The mean value of CIPA in group B was 18.68 units in the LVS with the confidence limits between 15.04-22.32 units; mean HR was 60.3 beats/min. with confidence limits between 52.0-68.6 beats/min. The mean CIPA value in group C was 17.20 units in the LVS with confidence limits between 13.13-21.27 units; mean HR was 71.5 beats/min. with confidence limits between 62.2-80.8 beats/min. The magnitude of CIPA was 250% greater in sleep deprived subjects in the combined groups B and C compared with the level in non-sleep deprived subjects in group A ($p < 0.01$). The corresponding 20% reduction in HR among sleep deprived compared with non-sleep deprived subjects was not statistically significant though consistent with an increase in vagal tone. There were no sex differences for either HR or CIPA within the three groups.

Correlation of CIPA with sleep stages

It is becoming increasingly clear that sleep is a dynamic and continuous process (21), so we looked for a correlation between sleep stages and CIPA. We found significant negative correlations between NREM sleep stages and group mean levels of CIPA in the respective stages among sleep deprived subjects in groups B and C (See Figures 2a and 2b). The correlation coefficients (r) were: $r = -0.88$ ($p < 0.05$) in group B, and $r = -0.9$ ($p < 0.05$) in group C. There was no significant correlation between group mean CIPA and NREM sleep stages in non-sleep deprived subjects in group A (See Figure 2c); $r = -0.2$ ($p < 0.40$). There was a significant difference between the group mean level of CIPA in sleep stage 0 compared with that in SWS stage 4 within subjects in group C ($p < 0.05$).

The group mean CIPA in sleep stage 0 was 19.25 units in the LVS with 95% confidence limits between 14.45-24.05 units, but it dropped to 10.0 units in the LVS with confidence limits between 6.4-13.6 units during SWS stage 4. Group mean values of CIPA were not significantly different in the various NREM sleep stages among subjects within groups A and B though there was a trend for a decrease in CIPA to be seen during SWS in group B subjects.

Figures 2a, 2b and 2c to be around here

DISCUSSION

The CIPA measured by the NeuroScope is specific as shown by its response to graded doses of atropine in this study. Such a response has been calibrated into units of a linear vagal scale in humans. Details of the derivation of this atropine based scale are reported elsewhere (14). The resting awake level of CIPA remains fairly constant throughout the day (18). Paradoxical increases of the CIPA following a very small dose of atropine, well illustrated in our study, is a known phenomenon which has been used in the treatment of cardiac tachy-arrhythmias (22). It is caused by atropine's central excitation of nucleus ambiguus in the medulla oblongata (23). The sensitivity of the NeuroScope technique to this central action shows that it can be used to monitor the cardiovagal output of these central neurons. Atropine abolished CIPA at a cumulative dose between 1.5-2.0 mg in this study, similar to the doses at which it has its maximum haemodynamic (24) and HR (25) effects. This technique is easy to apply in a clinical setting to enable the assessment of cardiovagal activity continuously. A further advantage of this method is that it allows a simultaneous and real-time measurement of both CIPA and HR and can be time-locked to the EEG record. This can facilitate

immediate on-line examination of their causal relationships and rapid changes in parasympathetic activity will not be missed or averaged out.

While these may be preliminary observations in a limited number of subjects, the findings in the combined population of sleep deprived subjects are consistent with previous observations and are statistically significant. Sleep deprivation was associated with a greater number of subjects having early onset SWS, consistent with the effects of sleep deprivation on sleep stage reported previously (26). None of our subjects was engaged in a regular fitness programme and all were judged to be in average physical condition thus excluding peak physical conditioning as a cause for elevation in CIPA (10). All the sleep deprived subjects in the present study had elevated CIPA in the awake epoch prior to the onset of sleep and there was a cut off level of CIPA for reaching SWS within the study period. This observation supports the findings by Zemaityte et. al. (10) that the progression of cardiac vagal activity in the different sleep stages observed throughout the night depended on the initial values of cardiac vagal tone. The authors speculated that this finding may be related to the effects of physical fitness. This is not a factor in our subjects.

The importance of a SWS deficit as a controlling mechanism of SWS in the homeostatic process S has been recognized (27,28,29). The homeostatic variable was so sensitive that intra-night rebound recovery of SWS was seen in the first 3 hours of recovery sleep after SWS deprivation (30). The elevated level of CIPA we observed in sleep deprived subjects immediately prior to sleep onset and its subsequent fall during SWS suggests a relationship between the cardiac parasympathetic activity and this homeostatic process S. It would require a further study of a large population to determine how close the relationship between CIPA and the homeostatic process is. Measurement of cardiac parasympathetic activity in stage 0 immediately prior to onset of sleep stage 1 may provide a non-invasive and easy means of assessing the homeostatic process S.

Circadian rhythms can be seen in a variety of physiological functions including the distribution of SWS and REM sleep, hormone secretion and body temperature (31). The

effect of circadian rhythms on cardiac vagal activity have not been measured directly but have been inferred from 24 hour study of HR variability (32). Otherwise changes in autonomic tone have been described with references to physiological states such as quiet wakefulness, SWS or REM sleep (9). We have addressed this issue by looking at the level of CIPA during quiet wakefulness throughout the day in one of the groups of subjects. No significant change was seen in the basal CIPA values before atropine was injected in these subjects. Further more, previous studies (33,34,35,36,37) have shown that slow wave activity declines over the first 3-4 non-REM sleep cycles irrespective of the circadian phase though the SWS could be influenced by the expression of REM activity (34). It is therefore unlikely that circadian rhythm affected the level of CIPA or SWS in our subjects.

The nucleus ambiguus in the ventrolateral medulla is the main site of origin of cardioinhibitory activity in the vagus nerve (38). There are close anatomical and functional relationships among neurons in the nucleus ambiguus, sympathetic, respiratory nuclei and the nucleus of the tractus solitarius (NTS) in the brainstem. They all constitute the medullary cardiorespiratory network (39). The NTS receives inputs from visceral organs (40,41), insula, medial frontal cortex (40), amygdala (42) and hypothalamic cell groups (43). The cardioinhibitory output from the nucleus ambiguus can therefore be influenced by different levels of the central nervous system (CNS) given the above anatomical connections. We propose that the elevated level of CIPA in sleep deprived subjects may be a reflection of the sleep homeostatic process. This process could affect the nucleus ambiguus through the connections with other parts of the CNS as outlined above. The significant correlation between levels of CIPA and the depths of sleep in sleep deprived subjects is consistent with the proposed hypothetical relationship between CIPA and a homeostatic process. We have also shown that CIPA decreased whenever SWS was achieved. Again this observation is consistent with our hypothesis that elevated levels of CIPA in sleep stage 0 reflects the homeostatic drive required to achieve SWS and was reduced to normal once SWS was reached.

Vaughn *et al.* (9) investigated 10 minute segments of sleep in non-sleep deprived subjects throughout the night and found that all neurogenic (including parasympathetic) influences on the heart decreased during SWS. Our results confirm the reduction in parasympathetic activity in SWS reported by these authors despite the small numbers of subjects and the fact we used the first ultradian period. We did not observe a reduction in the level of CIPA in non-sleep deprived subjects probably because too few of them achieved SWS within the first 90 minute period we studied.

The results of this study illustrate that the NeuroScope technique offers an opportunity to study vagal activity in sleep and its disorders. We were able to formulate an hypothesis based on statistically significant results, but obtained from a limited number of subjects. This will require confirmation in a further study of a larger population. We hope this study will introduce a novel method of measuring parasympathetic activity that can be time-locked with the EEG and this could facilitate the interpretation of autonomic effects in various sleep disorders particularly in cases where fast and transient changes in autonomic activity are suspected.

Legend Figure 1

The effect of the cumulative dose of atropine on resting CIPA in one of the five normal subjects. Note a paradoxical increase in CIPA following the initial small dose of atropine.

Legend Figure 2

The correlation between mean CIPA levels and NREM sleep stages in (a) sleep deprived normal subjects; (b) sleep deprived epileptic subjects and (c) non sleep deprived normal subjects.

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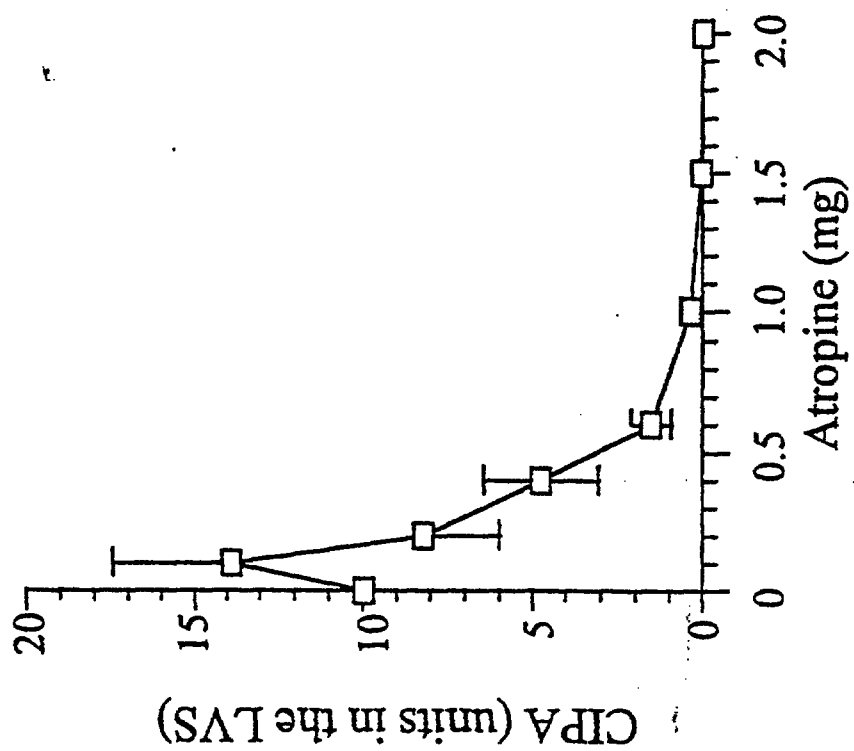
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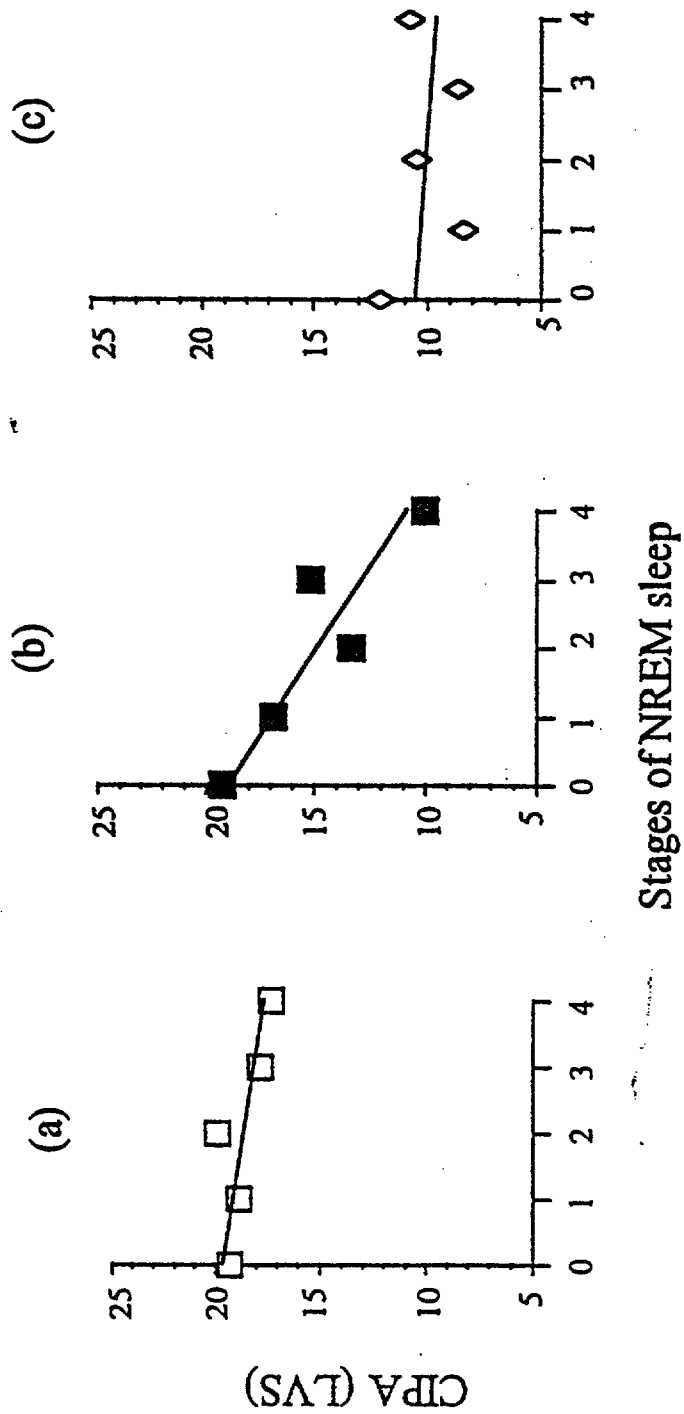
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Periodicity of a non-invasive measure of cardiac vagal tone during non-REM sleep in
non-sleep deprived and sleep deprived normal subjects

APPENDIX 3

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Running Title: Periodicity of vagal tone in sleep.

Summary

Cyclic alternating pattern (CAP) of microarousals reported in EEG studies is now regarded as an integrating mechanism for the different parts of the central nervous system including the autonomic system. A recently developed continuous index of cardiac parasympathetic activity (CIPA) can be time-locked to the EEG allowing the relationship between EEG and autonomic changes to be studied in sleep. Eleven normal subjects were studied for evidence of periodicity in CIPA in non-REM sleep, five of whom had been sleep deprived. Fast Fourier's transformation of the CIPA data confirmed its periodicity with four frequency peaks in the range 0.015-0.3 Hz. The frequency peaks previously reported as caused by respiration, Mayer waves and vasomotor thermo-regulatory activity were within what we called the α - and β -rhythms of CIPA. There was an additional very slow peak not previously described and we called it the γ -rhythm. It covered the frequency range below 0.03 Hz. The γ -rhythm was the largest of all peaks in CIPA rhythms and its magnitude increased further in sleep deprived subjects, but it invariably decreased during stage 4 sleep in both groups. Bursts of Alpha waves in the EEG recorded concomitantly with CIPA in stage 1 sleep were associated with both peaks and troughs of the γ -rhythm. These results support previous proposals that CAP in the EEG may be an integrating mechanism associated with functions of the central nervous system and we have shown here its relationship with CIPA. Since CAP can also be measured in CIPA, clinical exploitation of this phenomenon could include monitoring of epilepsy, studies of the effects of drug therapy and other sleep disorders. All these are known to affect cyclic patterns of sleep EEG.

Keywords: vagal tone; non-REM sleep; periodicity; cyclic alternating pattern, autonomic nervous system

Abbreviations: CAP = cyclic alternating pattern; CIPA = index of cardiac parasympathetic activity; FFT = fast Fourier's transform; LVS = Linear vagal scale; NREM = non-REM; SWS = slow wave sleep

Periodic changes in the autonomic nervous tone affecting physiological function during sleep have been reported by many authors for the greater part of this century. Periodicity in continuously monitored respiration was initially described by Reed and Kleitman (1925) and subsequently confirmed (Haab et al., 1957; Bulow, 1969; Kreiger, 1989). Medullary respiratory neurons are now regarded as cardiorespiratory neurons forming part of the autonomic system (Richter and Spyer, 1990). Periodic variability in arterial blood pressure in sleep has been correlated with the EEG in cats and dogs under experimental conditions (Bonvallet et al., 1954) and confirmed in humans (Lugaresi et al., 1971; Oswald, 1962; Coccagna et al., 1971). There are periodic EEG changes first described in drug induced coma by Fischgold and Mathis (1959), in neurosurgical patients (Lundberg, 1960; Cooper and Hulme, 1969) and in patients with pathological changes of Creutzfeldt-Jakob disease known to affect the cerebral cortex (Lundberg, 1960; Cooper and Hulme, 1969). Schieber *et al.* (1971) reported short episodes of what they called "*les phases d'activation transitoire spontaneés*" in the EEG occurring spontaneously every 4-5 minutes as well as after external auditory stimuli applied to their subjects throughout the night. The morphological appearances of these episodic EEG changes were identical whether induced by the external stimuli or having arisen spontaneously. The episodic changes were associated with a rise in the level of arousal and they occurred more frequently in superficial than in deep sleep. These episodic changes in the EEG in sleep subsequently called microarousals have also been shown to be present following sleep deprivation during the rebound recovery sleep when the Reticular Activating System tone is thought to be elevated (Halasz et al., 1979). Similar EEG changes were reported to be repetitive in a cyclic pattern (Terzano et al., 1985; Evans, 1992; Terzano et al., 1986) and were described by Terzano *et al.* (1985) as the cyclic alternating pattern (CAP), but are sometimes referred to as the arousal inhibition cycle (Evans, 1993). The CAP has been reported in sleep apnoea (Terzano et al., 1987), Creutzfeldt-Jakob disease (Terzano et al., 1981; Terzano et al., 1987) and it is noted to be present for significantly longer periods during sleep in patients with clinically active primary generalised seizures (Terzano et al., 1992).

METHODS

Subjects

With the approval of the local Ethics Committee, a total of eleven subjects were studied with their informed consent. Five had been deprived of sleep for 24 hours before the study. For these five subjects, a 90 minute recording of EEG and ECG was performed between 6 p.m. and 9 p.m. under similar conditions. Each subject was observed continuously by a technician who noted any changes in the behaviour of the subject.

The six non sleep deprived subjects had their EEG and ECG recorded between 6 p.m. and 12 midnight in the same room. On this occasion each subject was observed via a video camera and any changes in the behaviour of the subject noted from the video.

None of the subjects used in this study suffered from any neurological illness and they were not taking any medication and had not consumed any alcohol within 48 hours of the commencement of the study.

Neurophysiological Recording

The EEG of the sleep deprived subjects was recorded onto paper using a Nihon Kodan 4412 machine and Ag/AgCl electrodes placed in a standard international 10:20 montage. This enabled the record to be staged by a modified Rechtschaffen and Kales criteria (1968). The modification entailed the use of epochs with duration measured in 256 heart beats to facilitate fast Fourier's transform of the CIPA data which is time-locked to the EEG. The EEG record in each epoch was then sleep staged and those epochs which remained in the same sleep stage were used to study the periodicity in CIPA. The alpha activity recorded at electrodes A1-T3, T3-C3 and A2-T4, T4-C4 (Fig. 1) in those epochs classified as stage 1 sleep, was plotted together with the continuous record of CIPA against the same time domain to enable visual comparison (Fig. 2A). The presence of alpha activity at the electrode positions listed above for

The micro-arousals observed as the CAP are associated with cardiac autonomic changes during which a short lived increase in heart rate is the most commonly reported feature (Terzano and Parrino, 1991; Halasz and Ujjaszi, 1991). Previous studies of the cardiac autonomic changes have only used heart rate, R-R interval changes or blood pressure as means of obtaining a continuous measure of the autonomic influences on the heart. The relative contributions from sympathetic compared with parasympathetic (or vagal) activity have remained difficult to assess under these circumstances. Power spectral analysis of the periodicity in heart periods was suggested to be a useful technique for assessing the balance between sympathetic and cardiac vagal tone. However this technique requires data to be collected over a preset period, typically of the order of 5 to 10 minutes (Vaughn et al., 1995), which will average out any transient alterations in the autonomic tone. The method is also unable to give a continuous measurement of the activity of either of the two autonomic divisions to allow assessment of periodic changes of either the sympathetic or parasympathetic activity to the heart.

An index of cardiac parasympathetic activity (CIPA), or vagal tone can now be measured continuously (Little and Julu, 1995). This method was used successfully to show the extent of respiratory modulation of cardiac vagal tone in real-time in a dog with partial respiratory obstruction (Little and Julu, 1995). This study by Little and Julu revealed a spectrum of frequencies in the rhythmic variation in CIPA. The aim of our present study is to use this new technique to investigate possible periodicity in CIPA during early non-REM (NREM) sleep in sleep deprived and non-sleep deprived healthy subjects. We included sleep deprived subjects to optimise the chances of detection of periodicity in CIPA because of a previous report suggesting elevated tone of the reticular activating system in rebound sleep following sleep deprivation (Halasz et al., 1979). We have also observed an increase in CIPA following sleep deprivation in the first ultradian cycle of sleep in our laboratory (unpublished observation). A relationship will be sought between microarousals observed in the EEG during early NREM sleep and any periodicity in CIPA which will be time-locked to the EEG.

up to 50% of the epoch is indicative of sleep stage 1 (Rechtschaffen & Kales. 1968). Only the first 90 minutes starting from sleep stage 1 was examined so as to compare stage 1 and stage 4 sleep in the first ultradian cycle before the onset of REM sleep in each subject.

Fig 1 to be around here

For the non-sleep deprived subjects the EEG was recorded in a similar manner as in the sleep deprived using Ag/AgCl electrodes arranged in an international 10:20 montage. However, the signal was recorded onto a Telefactor video telemetry Beekeeper system (Telefactor Corporation, Pennsylvania, USA). Digital data was stored onto VHS long play video tapes and subsequently replayed on paper via a Nihon Kodan 4412 machine. The paper record was then staged in a similar manner as in the sleep deprived subjects. Again the alpha activity at electrodes A1-T3; T3-C3 and A2-T4; T4-C4 in those epochs classified as stage 1 sleep was plotted together with the continuous record of CIPA against the same time domain for visual comparison of periodicity (Fig.3A).

Measurement of the Index of Cardiac Parasympathetic Activity (CIPA)

The index of cardiac parasympathetic activity was determined from the analogue ECG output of the Nihon Kodan 4412 machine or from the digital to analogue converter in the Telefactor Beekeeper system. The analogue ECG signal was fed into the NeuroScope (Pontoppidan, Copenhagen, Denmark) for continuous measurement of the CIPA. The NeuroScope sampled the ECG signal at 5 kHz for accurate measurement of R-R intervals to obtain the heart periods used to decode the vagal influences on the heart. Decoding of vagal influences on the heart was done continuously within the machine at the end of every heart beat using a template of vagal effects on heart periods similar to a modification of the model reported by Katona *et al.* (1970). This template facilitates the detection of vagally induced delays in the cardiac cycle. The continuous value of CIPA generated beat-by-beat was then expressed in units of an atropine derived scale of vagal tone using the principles validated by

Julu (1992). In this scale, the specific vagal delays of the cardiac cycle measured in milliseconds by the NeuroScope is converted within the machine into arbitrary units of parasympathetic control of the heart. These units are linearly spaced within a linear vagal scale (LVS) (Julu, 1992) and they provide the best means of quantifying the continuous index CIPA. Examples of a continuous record of CIPA quantified using units in the LVS are shown in (Figs 2A and 3A).

Data analysis and statistics

The discrete nature of the CIPA data meant fast Fourier's Transform (FFT) was necessary to determine the major frequencies of its variation and to quantify the strength of periodicity in the data. Since this requires 2^n data points where $n > 2$, 256 data points were arbitrarily selected. These data points were the 256 consecutive cardiac cycles in the modified sleep epochs. This arrangement enabled the EEG and CIPA to be time-locked in the same time domain. A commercial software (Mathcard 5.0, Mathcard Inc. Massachusetts, USA) was used to perform FFT. A vector consisting of a spectrum of the powers of CIPA at different frequency domains (F_c) was obtained where F_c was expressed in Hz. The spectral power of CIPA obtained by FFT represented the strength of periodicity of CIPA at various values of F_c .

There are three distinct bands in the frequency domain of heart rate fluctuations; the high frequency band with its peak centered at the respiratory frequency, the medium frequency band with its peak centered around 0.15 Hz, and the low frequency band with its peak centered around 0.04 Hz (Akseirod et al., 1981). These distinct frequency bands are separated by borders of very low powers, typically less than 5% of total power. The frequency domain of CIPA was slightly different in that it had a major peak at a frequency lower than 0.03 Hz and the peaks at medium and high frequency bands were much smaller. To avoid confusion in the nomenclature, we called the frequency band centered at the respiratory or higher frequencies the α -rhythm. We then combined the low and medium frequency bands to make the β -rhythm and the frequency band below 0.04 Hz we called the γ -rhythm. The frequency with the largest power was the dominant frequency of fluctuation of CIPA in that epoch. The total power in each epoch was obtained by calculation of the total area under the frequency range 0-0.3 Hz.

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This frequency range was used as there were no peaks of vagal periodicity above 0.3 Hz in our sleeping subjects. The differences in mean total powers in various groups of subjects and between sleep stages 1 and 4 were compared using ANOVA and the 95% confidence intervals ($p < 0.05$) were used for statistical significance.

RESULTS

Subjects

The sleep deprived subjects were aged between 22-53 years, two males and three females (means $\pm$ SEM, years) 31.8 ± 7 in males and 35 ± 9.3 in females. Four of the subjects reached slow wave sleep (SWS) stage 4 whilst one subject only reached the light sleep stage 2.

The non-sleep deprived subjects were between 22-45 years old, three males and three females (means $\pm$ SEM, years) 36.3 ± 7 in males and 30.2 ± 7 in females. Three of the six subjects reached SWS stage 4 within the study period, while the rest only reached the light sleep stage 2.

Figure 2 to be around here

Periodicity of CIPA during sleep

Within each epoch studied, gross inspection of the data showed high amplitude slow frequency fluctuations in CIPA during stage 1 sleep in all the subjects. The amplitude of the slow frequency fluctuations of CIPA decrease in those subjects who reached Stage 4 sleep.

Three major rhythms could be identified following FFT of the CIPA data in stage 1 sleep where the fluctuation was optimum (see the powers of fluctuations below). The first group of high frequency peaks between 0.09 and 0.30 Hz we called the α -rhythm. The second group of peaks in the frequency band 0.04-0.05 Hz we called the β -rhythm. These two groups of peaks comprising α - and β -rhythms are caused by a mixture of respiratory, arterial pressure "Vasomotor" modulation of CIPA and thermoregulatory waves. A third group of low frequency peaks was in the frequency band below 0.04 Hz we called the γ -rhythm. There was a broad peak in this frequency band which covered 0.015 to 0.026 Hz. This broad peak in the γ -rhythm had the greatest magnitude compared with all the other peaks in each individual subject studied, indicating that the γ -rhythm is the dominant frequency of fluctuation in CIPA

during stage 1 sleep which is apparent visually in the time domain of the continuous records (Figs 2B and 3B). The γ -rhythm is slower than is generally accepted for vasomotor or Mayer waves (Guyton, 1991) and yet it is the dominant frequency of fluctuation in the cardiac vagal activity in sleep in all the subjects we studied. This γ -rhythm has a period varying between 23 and 40 seconds.

There was a significant difference in the total power of fluctuations in CIPA between sleep deprived and non-sleep deprived subjects in stage 1 sleep. The sleep deprived subjects had a total power between 129.9 and 221.9 with a mean of $187. \pm 17.8$ (LVS/ F_c) in the frequency band 0-0.3 Hz and the non-sleep deprived subjects had a total power between 37.7 and 67.7 with a mean of 49.6 ± 5.2 (LVS/ F_c) in the same frequency band, which was a greater than three times increase in total spectral powers for the sleep deprived subjects ($p < 0.001$). Comparing the total power in sleep stages 1 and 4 within each group, there was a significant decrease in the spectral power on reaching stage 4 in sleep deprived subjects only. The total power in sleep stage 4 in sleep deprived subjects was between 105.1 and 160.1 with a mean of 127.7 ± 12.7 (LVS/ F_c), a significant 32% drop in power compared with that in stage 1 ($p < 0.05$). In the non-sleep deprived group, the power in sleep stage 4 was between 28.6 and 50.6 with a mean of 41.8 ± 6.7 (LVS/ F_c) a non significant reduction in the power of CIPA in stage 4 sleep compared to stage 1 sleep. Overall, the fluctuation of CIPA in the whole epoch appeared to diminish in stage 4 sleep compared to stage 1 sleep reaching statistical significance in sleep deprived subjects.

Figure 3 to be around here

Respiratory modulation of cardiac vagal tone in sleep

The breathing frequency obtained by FFT due to respiratory modulation of CIPA in sleep deprived subjects in stage 1 sleep was (mean $\pm$ SEM) 10.2 ± 0.7 breaths/min and was 11.0 ± 0.9 breaths/min in stage 4 sleep. In non-sleep deprived subjects it was 12.5 ± 0.5 breaths/min in stage 1 sleep and 12.1 ± 0.9 breaths/min in stage 4 sleep. The sleep stages did not affect

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breathing rates in either the sleep deprived or non-sleep deprived subjects and there was no difference in the breathing rates of the two groups. As is evident from the powers of the respiratory peaks (Figs. 2B and 3B), the contribution of respiratory modulation to the total periodicity of CIPA is in fact quite small during sleep probably due to the meagre tidal volumes. This is probably because the amplitude of respiratory sinus arrhythmia depends to a large extent on the respiratory tidal volume (Hirsch and Bishop, 1981).

Sleep deprivation and cardiac vagal tone

The mean CIPA in the sleep deprived subjects was 20.9 ± 2.7 units in the LVS in stage 1 and 16.4 ± 1.6 units in the LVS in stage 4 sleep. The corresponding values for non-sleep deprived subjects were 5.4 ± 1.0 units in the LVS in stage 1 and 5.7 ± 0.9 units in the LVS in stage 4 sleep. There was a significant difference ($p < 0.001$) between those subjects who were sleep deprived compared to the non-sleep deprived with a more than three fold increase in CIPA in sleep deprived subjects during the two sleep stages we studied.

Association of Cortical EEG changes with CIPA in stage 1 sleep

A total of 41 alpha bursts (ABs) were seen in the five sleep deprived subjects in stage 1 sleep. The average number of AB being (means $\pm$ SEM) 8.2 ± 3.8 per individual in the epochs studied. Examples of the relationships between the AB and CIPA are shown below (Fig. 2A). One hundred percent of the ABs straddled either the peaks or troughs of the slow frequency rhythm observed in the continuous record of CIPA and the periods of the AB varied between 21 and 83 s with a mean period of 43 seconds.

Of the six subjects who were not sleep deprived, 114 ABs were identified with an average of 19.0 ± 5.2 per individual and 94.6% of the AB straddled the peaks or troughs of the slow frequency rhythm observed in the continuous record of CIPA. The periods of AB varied between 25 and 85 s with a mean period of 35 seconds (Fig. 3A). Six ABs not complying with

the above general rule occurred on the falling phases of the slow frequency rhythm of CIPA and no ABs were associated with external noises in any of our subjects.

DISCUSSION

This preliminary demonstration of periodic changes in CIPA in a limited number of subjects supports previous reports of periodicity in autonomic function assessed by analyses of heart rate, or blood pressure records (Koh et al., 1994). Power spectral analyses of heart rate had previously shown three broad peaks of frequencies in the band 0.04 to 0.5 Hz in dogs (Akselrod, 1988). Heart rate variability is believed to be secondary to fluctuating sympathetic and parasympathetic activity (Levick, 1991). The relative contributions from each of these two autonomic components towards the periodicity in heart rate was investigated using β -adrenergic and parasympathetic cholinergic blockade. The mid and high frequency (>0.09 Hz) peaks were abolished by β -blockade and cholinergic antagonism respectively but the low frequency (0.04-0.09 Hz) had variable responses depending on the physiological state at the time of the pharmacological intervention (Akselrod et al., 1981). The origin of low frequency periodicity in heart rate is still controversial, some authors have suggested that it could be due to vagal tone (Sleight et al., 1995; Saul, 1990). The index we have used for measuring parasympathetic activity of the heart is 94-97 % responsive to atropinisation demonstrating its very high specificity to the muscarinic receptor mediated autonomic activity in the heart (Julu, 1992). A similar technique was used to show the vagolytic effect of diabetes mellitus (Julu, 1993). The modification made in this present study was to enable continuous measurement of cardiac parasympathetic activity, which has shown here that the dominant frequency of fluctuation in this continuously measured vagal tone was in the low frequency band below 0.03 Hz. This result is in accord with that of Saul *et al.* (1990) who showed low frequency fluctuations (<0.03 Hz) in the heart rate and the arterial blood pressure. These were regarded

as quantifiers of autonomic responsiveness. The results of our present study support an important role of vagal input into the low frequency modulation of heart rate.

Fast Fourier transform of the continuous CIPA data revealed three broad rhythms which we have called α , β and γ . The α -rhythm contained the respiratory frequency, while the β -rhythm contained the Mayer wave and other frequencies consistent with vasomotor thermoregulatory activity described in previous studies (Koh et al., 1994; Akselrod et al., 1981). The γ -rhythm of CIPA was slower than all peaks previously known. This is consistent with a previous speculation by Koh *et al.* (1994) that there could be a slow vagal rhythm which is often overlooked. A similar slow peak has been identified following Fourier's analysis of CIPA in a dog with partial upper respiratory tract obstruction (Little and Julu, 1995).

It was interesting that sleep deprivation appeared to increase the fluctuations of CIPA in stage 1 sleep which decrease during slow wave sleep (stage 4). We have shown here that the slow frequency fluctuation in CIPA in stage 1 sleep corresponded to oscillations between α - and θ -activity in the EEG. The ABs in the EEG which can also be regarded as microarousals (Evans, 1993), were associated with either peaks or troughs of CIPA in the time domain. It is known that the number and frequency of these periodic microarousals visible in the EEG decreases as sleep deepens. We have therefore focused our study on the level of CIPA in the two extremes of NREM sleep, stage 1 at the beginning and stage 4 at the deep end. We made a direct comparison between the fluctuations in CIPA and microarousals in the time domain during the optimum sleep stage 1 when the fluctuation of CIPA and the number of microarousals were greatest. Our results also showed an elevation in CIPA following sleep deprivation confirming the results of previous studies (Halasz et al., 1979).

Recurrent arousals in stage 1 sleep with similar periodicity to the γ -rhythm of CIPA shown in this present study have been used as evidence of the involvement of a periodic mechanism in order to arrive at slow wave sleep (SWS) (Evans, 1993). These recurrent arousals in stage 1 sleep detectable as α -bursts (ABs) in the EEG are thought to be part of a proposed homeostatic process S required for attaining SWS (Borbely, 1982). We have shown in this present study that the variation in the autonomic tone represented by fluctuations in the level of CIPA is closely related to the α -bursts in the EEG during sleep stage 1. For these reasons, we

propose that the level of CIPA expressed in the units of the linear vagal scale could probably be used to assess the homeostatic process S. Current evidence suggests that this supposed homeostatic process S exerts an important control over SWS (Dijk et al., 1991; Beersma et al., 1987; Dijk et al., 1987; Borbely, 1982; Borbely et al., 1989; Brunner et al., 1993; Achermann et al., 1993; Borbely et al., 1981). Continuous measurement of CIPA during NREM sleep could provide a convenient means of monitoring or evaluating the effects of drugs or disease on the homeostatic process S.

The entrainment of autonomic function with the cyclic alternating pattern (CAP) indicated by ABs in the EEG during sleep stage 1 is well recognised (Terzano and Parrino, 1993). It is thought that the CAP is an integrative cerebral rhythm used by the brain to co-ordinate the general biological functions with its own internal levels of activation (Terzano and Parrino, 1993). It is also further recognised that the CAP may be the means through which various physiological states are integrated into longer lasting ultradian, circadian and infradian rhythms (Terzano and Parrino, 1993). These would suggest an important functional role for the CAP and a simpler method of monitoring it by measuring CIPA without the cumbersome EEG may prove useful in future experiments.

It is during NREM sleep that interictal abnormalities are seen with the greatest frequency in epilepsy (Kellaway, 1985). The importance of the CAP in patients with epilepsy is also now evident. The period of the CAP can be prolonged when they are associated with epileptic paroxysms. As a corollary, the majority of all interictal epileptiform EEG discharges occurring during NREM sleep were observed in association with the CAP (Terzano et al., 1989). A similar observation has also been reported for focal interictal epileptiform EEG discharges (Terzano et al., 1991). A great majority of nocturnal seizures with motor signs occur during the periods of sleep associated with CAP (Terzano et al., 1991). All of these observations suggest that CAP may not be just an integrating mechanism, but could also have a role in epilepsy. The simple method of continuous monitoring of the autonomic fluctuations associated with CAP reported in this present study may stimulate interest for future studies of its physiological role in sleep and other clinical conditions such as epilepsy.

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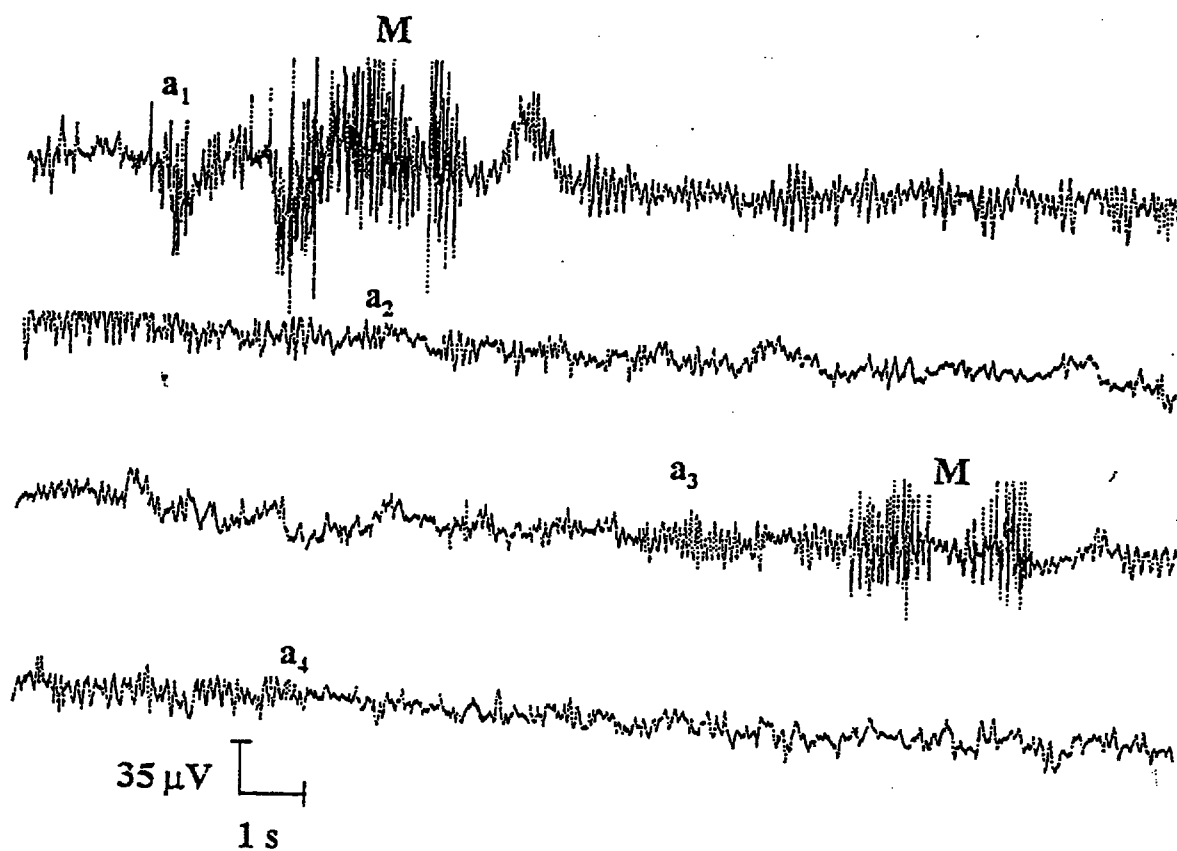
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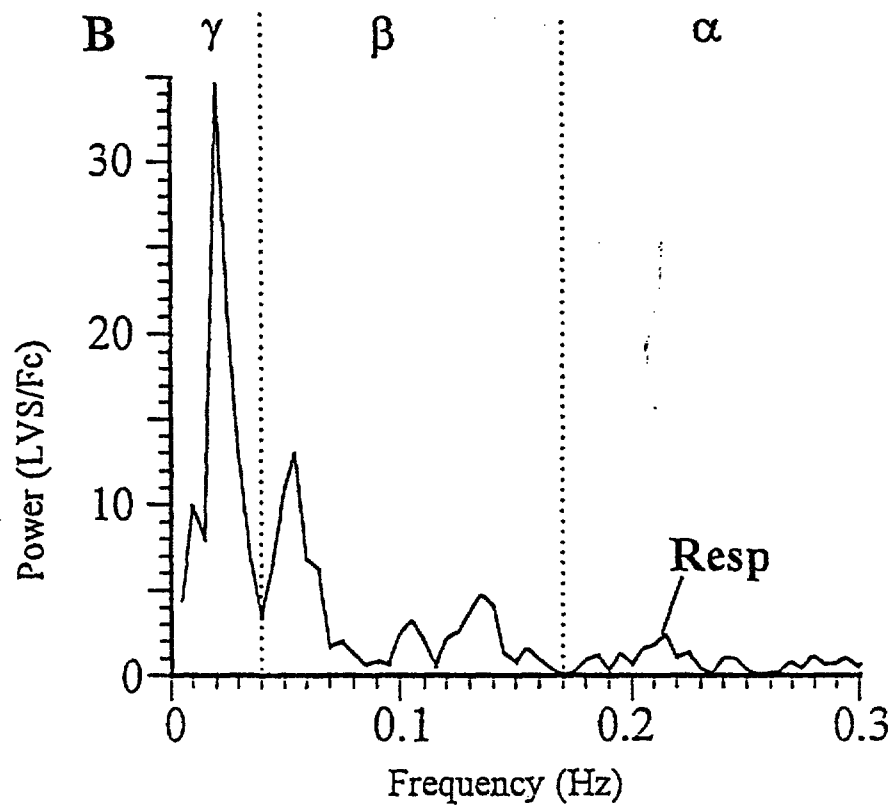
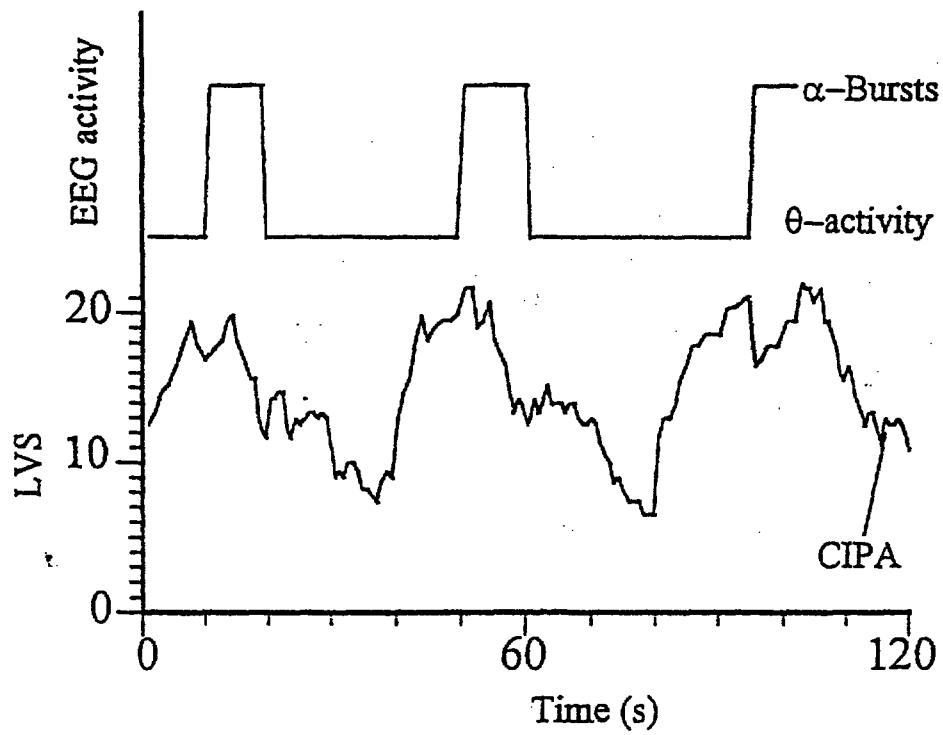
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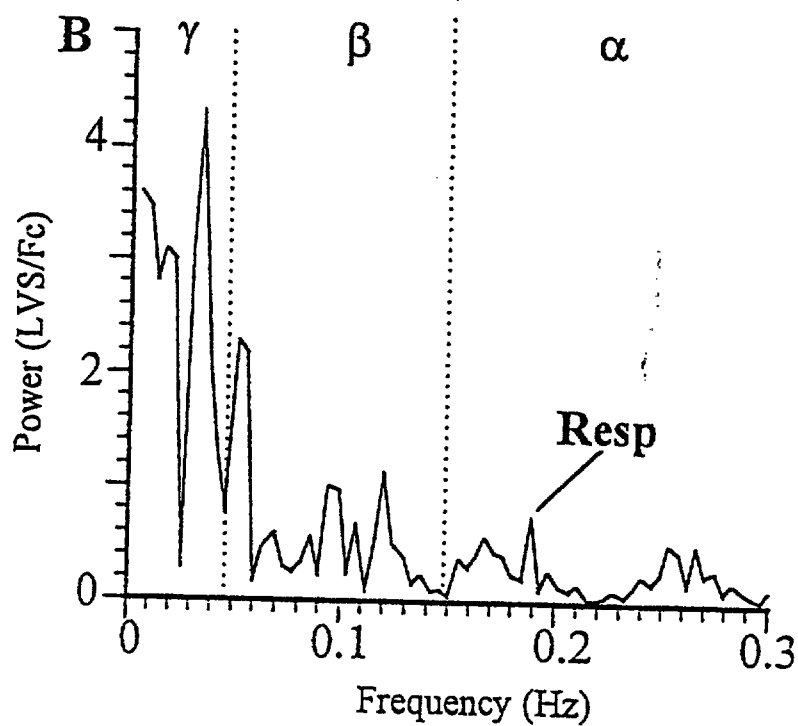
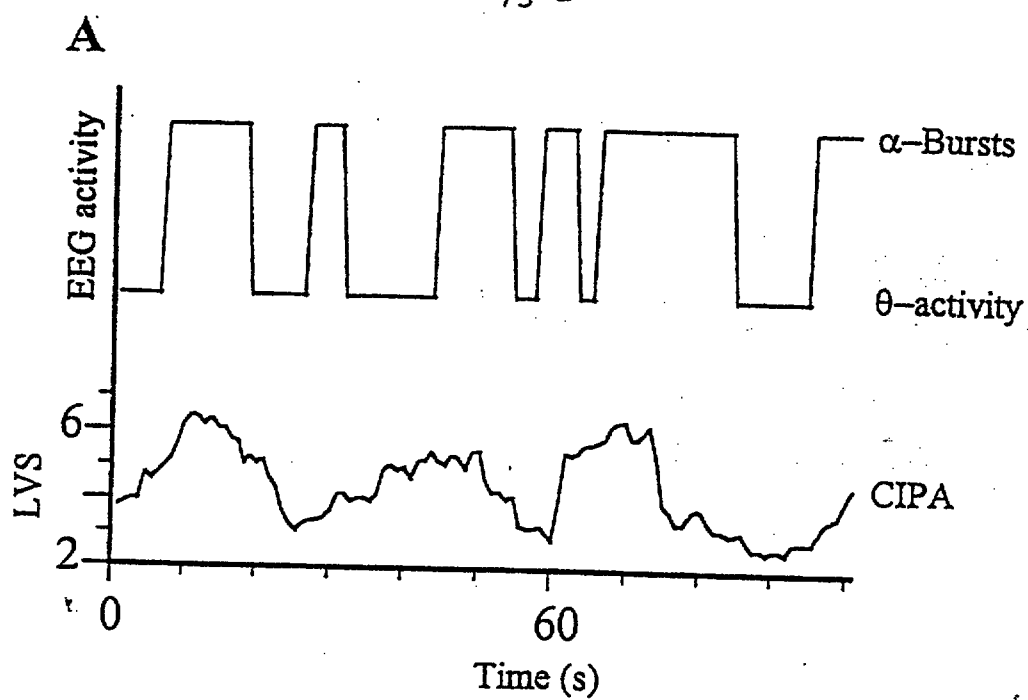
Fig. 1 A 64 s section of continuous EEG record at the electrode position (A2-T4) in stage 1 sleep in a sleep deprived male subject illustrating two spontaneous alpha bursts (AB) at a_1 - a_2 and a_3 - a_4 with intervening theta activity where the alpha activity drops out without any vertex sharp waves. The superimposed muscle activity indicated as M in the record is often seen at the onset of each AB, a sign of increased level of arousal in the subject. α -Waves have variable frequencies between 8-13 Hz and peak-to-peak amplitudes of 20-50 μ V. Paper speed was 1 cm/s, amplifier gain was at 70 μ V/cm, time constant was 0.3 sec with high pass filters set at 70 Hz.

Fig. 2 (A) The relationship between alpha bursts (AB) and the continuous record of cardiac parasympathetic activity (CIPA) in a sleep deprived male subject in stage 1 sleep. The AB is in phase with the peaks of CIPA and there were no AB at the troughs. Note that CIPA measured in units of the linear vagal scale (LVS) varied between 6-21 units with a periodicity of about 40 s. The AB had a similar periodicity. (B) Fast Fourier's transform of the continuous CIPA data into its frequency components showing various peaks within three major rhythms. The high frequency band between 0.17-0.3 Hz, we named the α -rhythm has respiratory frequency marked (Resp) in the record as its largest peak. The medium frequency band between 0.04-0.17 Hz we named the β -rhythm has Mayer waves in the range 0.1-0.14 Hz and thermoregulatory vasomotor waves in the range 0.04-0.05 Hz. The largest peak in the β -rhythm was that of the thermoregulatory vasomotor waves. The low frequency band below 0.04 Hz, we named the γ -rhythm has a peak at 0.02 Hz. This is larger than any other peak in other rhythms and represents the periodicity of 40 s in CIPA illustrated in (A).

Fig. 3 (A) The relationship between alpha bursts (AB) and the continuous record of cardiac parasympathetic activity (CIPA) measured in units of a linear vagal scale (LVS) in a non-sleep deprived male in stage 1 sleep. The ABs came at the peaks and troughs of the CIPA record. Note that the level of CIPA varied between 3-7 units in the LVS with a periodicity of nearly 35 s. The periodicity of the ABs was nearly half that of CIPA at about 17 s. The ABs corresponding with the peaks of CIPA were of longer duration than those corresponding with the troughs. (B) Fast Fourier's transform of the continuous CIPA data into its frequency components showing peaks within three major rhythms. The high frequency band between 0.15-0.3 Hz we named the α -rhythm has the respiratory frequency marked (Resp) in the record as its largest peak. The medium frequency band between 0.045-0.15 Hz we named the β -rhythm includes the Mayer waves in the range 0.09-0.13 Hz, and the thermoregulatory/vasomotor waves in the range 0.045-0.06 Hz. The low frequency band below 0.04 Hz we named the γ -rhythm had the largest peak at 0.035 Hz. This is larger than all other peaks in the three rhythms and represent the main periodicity of nearly 35 s in CIPA illustrated in (A)

Figure 1

Figure 2

Figure 3

Immaturity of medullary cardiorespiratory neurones leading to inappropriate autonomic reactions as a likely cause of sudden death in Rett's syndrome

Editor.—Rett's syndrome is a cause of intellectual disability with frequent respiratory dysrhythmia. In a survey in Great Britain, we identified episodic hyperventilation in 75%, apnoeic attacks in 70%, non-seizure vacant spells in 77%, and epileptic seizures in 70% of 191 classic cases. Of all reported deaths from Rett's syndrome, 25% were sudden and unexpected. Certain groups of interacting neurones in the medulla perform autonomic and respiratory functions that can be measured non-invasively. With informed consents and the approval of the local ethics committee, we measured the autonomic reactions to hyperventilation in Rett's syndrome and age matched controls while monitoring their respiration to understand the interactions between medullary autonomic and respiratory neurones.

Breathing movements were monitored using a resistance plethysmograph tied around the chest. Sympathetic activity related to the mean arterial blood pressure (MAP) was measured continuously and non-invasively using the Finapres. Cardiac vagal tone (CVT) was also measured continuously and non-invasively from the responses of heart rate to spontaneous baroreflex. These were converted into atropine derived units of a linear vagal scale (LVS),² by a machine, the NeuroScope. All measurements including heart rate were integrated and stored beat-by-beat into a microcomputer.

In eight control girls aged 4–11 years, mean (SEM) CVT was 10.5 (0.9) units in the LVS, MAP was 94.6 (6.4) mm Hg. During voluntary hyperventilation, vagal tone responded briskly and successfully corrected the raised MAP (fig 1). In six girls with Rett's syndrome of the same age group, MAP was 78 (4.33) mm Hg during quiet rest. Vagal tone was 3.6 (0.7) units in the LVS, 65% lower than in controls ($p < 0.001$, t test), but similar to the

3.0 (0.6) units previously reported in quiet neonates.³ During spontaneous hyperventilation, vagal tone responded briskly, but failed to correct a grossly raised MAP (fig 1). Vagal tone was invariably withdrawn at the height of sympathetic activity during hyperventilation. This sympathovagal imbalance bears the risk of cardiac arrhythmias in Rett's syndrome, a possible cause of sudden death.

We suggest that medullary cardioinhibition is immature in Rett's syndrome. Although neuropathological studies have shown immaturities in other areas of the brain,⁴ this is the first functional evidence of immaturity which is potentially useful for diagnosis and management of Rett's syndrome.

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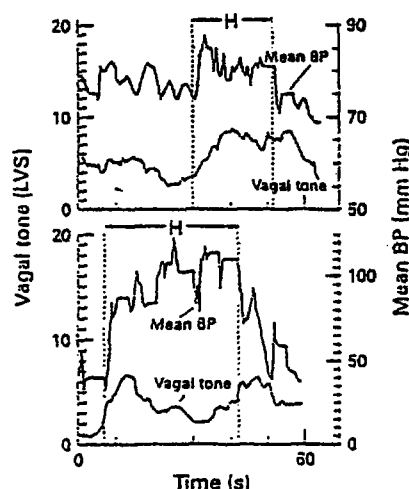


Figure 1 Continuous and concurrent records of mean arterial blood pressure (BP) and a measure of cardiac vagal tone expressed in units of a LVS. The upper pair of traces are examples obtained from a normal 8 year old girl to show the reaction to voluntary hyperventilation (H). The lower pair of traces are examples obtained

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Continuous measurement of the cardiac component of arterial baroreflex (CCBR) in real time during isometric exercise in human volunteers

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Cardiac response to specific arterial baroreceptor (ABR) stimuli such as neck suction or α_1 -adrenergic excitation by phenylephrine can be quantified as $\Delta RR/\Delta BP$, where ΔRR is the change in ECG R-R intervals associated with the change in blood pressure ΔBP (Fritsch *et al.* 1989). These are exaggerated stimulations of the ABR during which ΔBP is expected to be directly responsible for any changes in the heart rate (HR) causing the ΔRR . The quantity $\Delta RR/\Delta BP$ is often the slope of a curve of R-R intervals plotted against the respective systolic BP (SBP) and is referred to as the baroreflex sensitivity. Since the threshold pressure to excite the ABR is ~ 50 mmHg (Qu & Stuesse, 1991), the normal range of mean arterial BP close to 100 mmHg would cause a continuous discharge, but in type I receptors this is prevented by their rapid adaptation and poor response to slow changes in BP (Seagard *et al.* 1990). However, during the ejection period in systole the ABRs are briefly excited above the baseline level by the rapid rise in pressure from 80 to 120 mmHg within 60 ms in a typical cardiac cycle. It takes nearly 240 ms for the fastest nerve impulses to travel from the ABRs to sino-atrial node (SA node) through the vagus nerve (Eckberg, 1976). This means the effect of a brief excitation of the ABRs would arrive at the SA node in time to be registered in the period of the cardiac cycle following the stimulus (Eckberg, 1976). It follows that the cardiac response to the ejection pressure of a given cardiac cycle (i) can be quantified as $\Delta RR_{i+1}/\Delta SBP_i$, where $\Delta SBP_i = SBP_i - SBP_{i-1}$ and $\Delta RR_{i+1} = RR_{i+1} - RR_i$. Baroreceptor control of the heart can be modelled as a closed loop feedback system with a delay line in the sympathetic nervous system that makes it resonate at ~ 0.1 Hz (DeBoer *et al.* 1987). This means the effect of one perturbation is diffused over a period of at least 10 s, which is twelve cardiac cycles if the HR is 72 beats min^{-1} . A better quantification of the effect of one ejection pressure on R-R intervals would be a triangular weighted average of eleven cardiac cycles, five cycles on either sides of (i) to achieve a zero phase shift. This arrangement can be used as a moving average to achieve a continuous measurement of the cardiac response to systolic ejection pressures. We have implemented the above principles to assess the CCBR in real time during isometric exercise in a microcomputer-based set-up. Arterial SBP, mean BP (MAP) and diastolic BP (DBP) are measured non-invasively in one finger held at the level of heart using the FinapresTM (Ohmeda, Eagleswood, USA) and logged beat-by-beat into a microcomputer. The ECG R-R intervals are measure using the NeuroScope (Pontoppidan, Copenhagen, Denmark) and logged into the microcomputer simultaneously with the BPs. The beat-to-beat SBP, MAP and DBP are displayed on the screen together with their respective RRs. The HR is calculated from the RRs. A moving average of CCBR is also displayed on the screen while maintaining the temporal relationships with the beat-to-beat BPs and HR (Fig. 1). The CCBR obtained by this method is numerally very close to the baroreflex sensitivity obtained from the slope of BP

APPENDIX 5

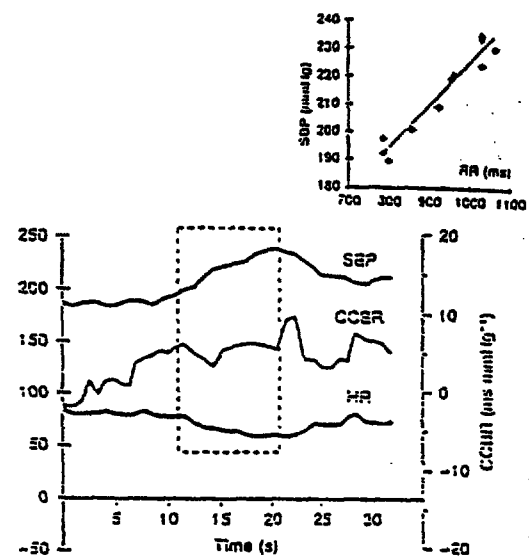


Fig. 1. Segment of a computer screen plot showing temporal relationships between beat-to-beat SBP, HR and CCBR following phenylephrine injection (300 μg i.v.) at time 0. Interrupted lines enclose the phase of SBP rise associated with decreasing HR. The RR versus SBP plot (inset) has a correlation coefficient of 0.98, and slope of 6.1 ms mmHg^{-1} . CCBR was fairly steady during phase with an average value of 5.3 ms mmHg^{-1} .

The subject sits in front of a table and holds a spring-loaded apparatus calibrated in Newtons for measurement of grip force. The maximum sustainable grip force is measured and 50% of this is used for isometric contraction of the muscles of the forearm. The isometric contraction is achieved through a visual feedback loop in which a needle pointer indicates the required force and the subject is asked to maintain that grip without drift or sway of the pointer. This can be sustained for up to 3 min. The beat-to-beat BPs and HR increased gradually but steadily during isometric exercise, while the recovery at the end of the exercise was immediate in all our preliminary experiments using this set-up. There were no obvious changes in pulse pressure during isometric exercise in these experiments. The CCBR decreased to near zero faster than the increases in HR or SBP with negative values at times during isometric exercise and the recovery at the end of the exercise was also immediate. These observations are consistent in all our experiments so far and we would like to demonstrate it live in as many volunteers as possible during the meeting. The set-up could be useful for the study of temporal relationships of BP, HR and CCBR, three major parameters involved in cardiovascular regulation during exercise.

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*Draft*APPENDIX 6**REAL-TIME MEASUREMENT OF CARDIAC VAGAL TONE IN CONSCIOUS DOGS.****Little C.J.L., Julu P.O.O., Hansen S., Reid. S.W.J.****Introduction:**

In health heart rate is determined by the intrinsic rate of spontaneous depolarisation of the sino-atrial node modified by the autonomic nervous system. Sympathetic influences accelerate heart rate, parasympathetic discharges cause deceleration, but at rest there is a tonic level of activity in each of these components; heart rate depends on the interplay between these (Hainsworth, 1995). The heart is not a continuous pump, each heart beat is a discrete event so that the R-R wave intervals (heart periods) vary as sympathetic and parasympathetic influences affect each beat. In man and the dog at rest parasympathetic (vagal) tone predominates. The relationship between R-R wave interval and the frequency of cardiac vagal stimulation is linear, an increment in the activity of vagal efferents prolongs the R-R interval by a fixed value independent of the initial R-R interval (Parker and others, 1984; Mary and Hainsworth, 1993).

Cardiac vagal tone is reflexly generated through baroreceptor stimulation (Keele, and others 1982). These baroreflexes are mainly responsible for resting cardiac vagal tone in the normally breathing animal although chemoreceptor afferents and other reflex inputs associated with breathing, for instance, may modify vagal outflow (Jordan, 1995; Daly, 1995). Baroreflex modulation of sinus node function is strongly influenced by the timing as well as the intensity of the stimulus (Brown and Eccles, 1934; Eckberg, 1976) but under physiological conditions the stimulus is the rapid rise in blood pressure during the ejection period of ventricular systole which occurs at a relatively fixed time after each heart beat. The latency of this reflex is constant and short compared to the length of the cardiac cycle, averaging 160-180 msec in the dog and 240msec in man (Katona and others; 1970 Eckberg, 1976). It follows that the effect of brief arterial baroreceptor stimulation arrives at the sinus node in the period of the cardiac cycle following the stimulus and is thus synchronised to the preceeding pulse. Moreover, at normal heart rates this volley of vagal impulses arrives at the sinus node at the stage of

the cardiac cycle during which it seems to be particularly sensitive to this stimulus (Eckberg, 1976). Thus, baroreceptor stimuli acting via cardiac vagal tone cause a delay in the cardiac cycle or cycles that follows that stimulus (deBoer and others, 1987). The delay in the cycle(s) induced by baroreceptor stimulation is proportional to the baroreceptor stimulus strength and duration (Eckberg, 1976). Volleys of vagal nerve impulses liberate acetylcholine (ACh) from synaptic vesicles and this ACh is added to the ACh remaining at the sinus node at the end of the previous heart period, but ACh concentration also declines exponentially due to degradation by acetylcholinesterase (Mokrane and others, 1995). In effect the vagally mediated prolongation of heart period is proportional to the concentration of acetylcholine at the sinus node (Katona and others, 1970). Thus the sinus node integrates (or adds up) the vagal impulses.

Rapid changes in heart rate are caused by changes in the level of parasympathetic tone. Sympathetic influences affect heart rate both directly and reflexly via changes in peripheral resistance but both of these controls act slowly in comparison to cardiac vagal tone (Hainsworth, 1995). Baroreceptor control of heart rate can be modelled as a closed loop feedback system with a delay in the sympathetic nervous system which makes it resonate at $\sim 0.1\text{Hz}$ (deBoer and others 1987). In a classical study of the firing rate of cardiac vagal efferent (CVE) fibers on the heart period performed in dogs Katona and others (1970) showed that it was possible to predict the heart period from CVE activity even while the sympathetic nerves were intact. These authors further demonstrated that a simple electronic model successfully described moderate changes in heart period due to changes in vagal nerve activity. It follows that it should be possible to deduce the instantaneous activity in the cardiac vagal nerves by evaluation of the heart period.

The NeuroScope is a novel electronic device which has been designed to offer an objective real-time measure of instantaneous cardiac vagal tone based on these physiologic principles. The instrument detects pulse synchronised delays in the onset of successive cardiac cycles, quantifies these delays in milliseconds and transforms them into measures of cardiac parasympathetic activity. Cardiac vagal tone introduces delays in the onset of cardiac cycles (Eckberg 1976) which can be detected with the aid of a template (Katona and others, 1970). The detection procedure involves accurate sampling of the E.C.G. to obtain a high resolution time domain of the R-R wave intervals; pulse synchronised delays, which are

specifically caused by cardiac vagal tone, are then obtained through phase demodulation of the time domain. Results are displayed on a computer screen in real time against an arbitrary but linear scale, the Cardiac Index of Parasympathetic Activity (CIPA) which was derived from human volunteers during atropinisation according to principles set out by Julu (1992). On this scale 10 represents the average cardiac vagal tone in supine resting adult male persons when breathing quietly, whereas 0 represents no impulses travelling through the vagus nerves to influence heart rate (Julu, 1992a). It follows from the above that the Neuroscope is only of use for the evaluation of sinus rhythm.

Hitherto publications arising from this technique have largely appeared in the clinical literature. Here we describe a validation of the technique in dogs by evaluation of the effect of graded doses of atropine on the CIPA index.

Material and Methods:

Ten healthy mature male beagles exhibiting sinus heart rhythm were used for this study. All experiments were performed in fully conscious dogs between the hours of 10am and 4pm following an overnight fast. Each dog had been trained to stand for long periods in a semi-supported standing position. They were familiar with the laboratory environment and the investigators. Adhesive silver/silver chloride ECG electrodes were fastened to the limbs and a 20G intravenous catheter was placed in the cephalic vein of a forelimb for delivery of atropine and/or saline. Beat-by-beat R-R wave intervals and CIPA values were recorded by downloading the ECG signal to the NeuroScope and from there to a laptop computer where these data were also stored automatically.

After instrumenting, each dog was allowed to rest for at least 2000 heart beats. Following this, as a test of intact cardiac vagal tone, the oculocardiac reflex was induced by pressing on the closed eyelids for a period of not less than 20 seconds (Gandevia and others, 1978). A further rest period of not less than 800 heart beats was allowed before the experiment continued and the last 500 beats of this rest period were designated as baseline. Six aliquots of 0.01mg/kg of atropine were given to each dog by intravenous injection at ten minute intervals to a cumulative dose of 0.06mg/kg. Each injection was followed by a 1.5ml flush of saline. Data were collected continuously during the study and for ten minutes after the final dose. At the end of the study period eyelid pressure was repeated to induce evidence of residual cardiac vagal tone. On a separate occasion the same dogs were prepared and studied in exactly the same way but the atropine injection was replaced with a similar volume of normal saline solution. The studies were performed in random order according to a predetermined schedule.

All data were examined visually during data collection throughout the recording to ensure that the dogs were exhibiting a sinus rhythm. For the purposes of data analysis beats which were not of sinus origin were excluded. The 50 heart beats prior to each injection and the subsequent 50-100 beats were also excluded from data analysis to avoid effects from handling of the dogs and to allow for equilibration. For each dose aliquot in each dog all other data were subject to further analysis.

Statistical Methods

In order to assess whether there was a significant difference between the responses of the animals between the two treatments the resting and experimental data were analysed using two factor analysis of variance (ANOVA) techniques with repeated measures. The factors were *dog*, *treatment* and *dose*, where *treatment* and *dose* were regarded as fixed effects and *dog* as a random effect. For the resting data the analysis was performed on the mean CIPA values for each segment of 250 heart beats for each dog and for each treatment. For the experimental data, because the heart rate changed and episodes of second degree heart block were sometimes observed during administration of atropine, the analysis was performed on the mean of the CIPA values for each animal at each dose for each treatment. The analysis of the experimental data was also performed on normalised data where the CIPA values were expressed as a percentage of the baseline value at dose 0 (i.e. the 500 beat time-frame immediately preceding the injections of atropine or saline). Finally, ANOVA was applied to the treated (Atropine) group alone and a multiple range test was used to identify at which dose the CIPA values were significantly different. Significance was set at 5% for all analyses.

Results:

Resting and Baseline data:

All dogs exhibited sinus rhythm during the resting and baseline recordings and only nine non-sinus beats were identified in three dogs during this period. The CIPA values recorded from the dogs in this study were generally much higher than those recorded from healthy human subjects. The mean (standard deviation) of the mean CIPA values over 500 heart beats for the ten dogs at baseline, prior to the injection of saline and atropine were 38.9 (16.8) and 43.8 (19.1) units respectively. Table 1 provides a summary of the mean and standard deviation of these measurements for each dog at baseline prior to the injection of saline or atropine. During these recordings CIPA values and R-R interval length in each dog varied widely over a relatively short time-frame. In some, but not all, individuals respiratory modulation of the R-R interval length, was marked.

CIPA values generally rose as R-R interval length increased and as variability in the R-R wave intervals rose. Over segments of 250 cardiac cycles in the resting period prior to injection of either saline or atropine the average CIPA value for each dog tended to be relatively stable and characteristic for that dog although dog to dog differences were not explored statistically.

In all dogs induction of the oculocardiac reflex by the application of pressure to the eyes was associated with lengthening of the R-R interval and an increase in R-R wave variability, beat-by-beat CIPA values rose steeply to reach a peak within 15-20 seconds in all dogs.

Statistical analysis of the resting data in the ten dogs showed no systematic or significant differences between the measurements preceeding atropine or saline injection. Moreover systematic and statistically significant differences were also absent over the eight consecutive 250 beat segments comprising this resting period.

Atropinisation:

Administration of a series of atropine boluses to these dogs systematically reduced the beat-by-beat CIPA values in all cases. After a cumulative dose of 0.06mg/kg this index was all but obliterated so that the CIPA values had fallen to less than 2.3% of the baseline value in all cases (mean 1.3%, SD 0.7%, range 0.3 - 2.3%) (Figure 2). Variability of

Discussion:

Atropine is a competitive muscarinic receptor antagonist which prevents the action of acetylcholine on the sino-atrial node of the heart.

Physiologic responses to parasympathetic (vagal) nerve impulses are thereby attenuated or abolished. Since the peripheral vasculature is virtually devoid of muscarinic receptors the effects of atropine on heart rate account for all the important effects of this drug on the cardiovascular system. In veterinary clinical use atropine is generally used in the dose range 0.01-0.04mg/kg (Bonagura and Muir, 1992). However, numerous experimental studies have been performed using this drug in conscious and anaesthetised dogs in doses ranging from 0.0003 - 1.5 mg/kg (Kottmeier and Gravenstein, 1968; Katona and others, 1977; Muir, 1978; Halliwill and Billman, 1992; Chassaing and others, 1992; Vicenzi and others, 1995). These studies have established that very small doses of atropine (< 0.01 mg/kg) slow the heart, possibly due to a central vagal stimulating action although the mechanism is controversial (Kottmeier and Gravenstein, 1968; Katona and others, 1977; Julu, 1992). In conscious dogs vagal effects on the heart are reported to be abolished at dose rates around 0.04 mg/kg but certain anaesthetic agents such as morphine and α chloralose seem to enhance cardiac vagal tone. Very high doses of atropine (> 0.2 mg/kg) appear to cause excess tachycardia, an effect which may be due to ganglionic blockade (Chassaing and others, 1992; Vicenzi and others, 1995).

In the present study cumulative intravenous atropine injections caused a dose dependant reduction in the CIPA values recorded from conscious dogs, and at the maximum dose utilised, this index was virtually obliterated in all cases to a mean value of only 1.3% of the resting value. This observation indicates that CIPA is a highly selective and dependable index of cardiac vagal tone. In keeping with this observation the CIPA values generated on a beat-by-beat basis rose steeply when cardiac vagal tone was increased by the application of digital pressure to the eyes, a well established vagal manoeuvre. This phenomenon was abolished by atropinisation. At rest CIPA values recorded from these dogs were seen to vary widely over a short time-scale. This would be consistent with known physiological concepts of the beat-by-beat control of the cardiovascular system (deBoer and others 1987).

In the present study as atropinisation proceeded in these dogs the mean R-R wave interval fell. This observation was expected but was of only

limited value as an index of cardiac vagal tone because both the initial and final mean R-R wave intervals varied quite substantially between dogs. The final value varied from 30.3 - 67.3% of that recorded at baseline (mean 51.5%, SD 11.5%). A dose dependant reduction in the standard deviation of the R-R wave interval with cumulative atropinisation was also observed in this experiment. However at the maximum dose of atropine given this index was not completely abolished such that the mean value recorded from the ten dogs represented 10.6% of the baseline figure. Moreover very substantial differences were observed between dogs. This observation was to be expected because although the main contributor to the variability of the R-R wave interval is cardiac vagal tone cardiac sympathetic tone will also affect this, particularly since this index was measured over a period of several minutes. The heterogeneity of response of this index between dogs can be explained by differences in the relative dominance of sympathetic and parasympathetic autonomic tone between individuals.

Various methods for the evaluation of vagal effects on heart rate have been described previously. Respiratory modulation of heart rate, respiratory sinus arrhythmia (RSA), occurs by modulation of cardiac vagal tone (Katona and Jih, 1975; Daly, 1995; Hedman and others, 1995; Swenne and others, 1996). Whether RSA is secondary to respiratory blood pressure variability or vice versa is controversial but since these components are connected through a closed feedback loop either variable could lead the other (Akselrod et al 1985; deBoer et al, 1987; Laude and others, 1993; Bernardi and others, 1994; Daly, 1995). In dogs and people under controlled conditions RSA has been shown to be closely correlated with parasympathetic control (Katona and Jih, 1975, Eckberg, 1983; Fouad and others, 1984). On the other hand it has long been known that over a range of breathing frequencies fluctuations in heart rate are not necessarily in phase with respiration (Angelone and Coulter, 1964; Laude and others, 1993). RSA in co-operative human patients can be maximised by asking them to breathe deeply at a slow rate often with the aid of a timing device such as a metronome. In conscious freely behaving animals by contrast rate and depth of breathing can vary substantially from moment to moment particularly in dogs who tend to pant rapidly when warm, stressed or excited. Moreover, because breathing rate and depth differ, it is difficult to make meaningful comparisons between individuals or over time.

Another approach to the issue of non-invasive assessment of cardiac vagal tone is to study heart rate variability (HRV). This latter approach has generated numerous publications but interpretation of the results viz- a viz cardiac vagal tone is difficult. There is agreement that high frequency variation in heart periods is solely associated with parasympathetic tone (Akselrod and others, 1985; Pomeranz and others, 1985; Sleight and Casadei, 1995). Lower frequency heart rate variability seems to be associated with changes in both sympathetic and parasympathetic tone interacting with each other; additional mechanisms such as thermoregulation and the renin-angiotensin-aldosterone system have also been implicated, particularly for rhythms with frequencies below 0.1Hz (Akselrod and others, 1985; Pomeranz and others, 1985; Sleight and Casadei, 1995; Sleight and others 1995). Evaluation of HRV to elucidate the components of autonomic control is crucially dependant on stationary conditions (Akselrod, 1995; Sleight and Casadei, 1995; Minors and O'Grady, 1997) which are difficult to achieve in freely behaving animal subjects. This necessity for stationary conditions necessarily hampers studies of reflex modulation of heart rate.

In contrast to measures of RSA and HRV as indices of cardiac vagal tone the Cardiac Index of Parasympathetic Activity is generated in real-time without the active co-operation of the subject. The measurement appears to be a specific and sensitive marker of beat-by-beat cardiac vagal tone which varies in response to well established vagal manoeuvres. Moreover, because the index is displayed in units against a linear scale, comparisons of cardiac vagal tone between individuals and over time are facilitated.

There is a growing interest in the measurement of cardiac vagal tone in the clinical arena to facilitate the recognition of cardiac failure and the identification of patients at risk of mortality following myocardial-infarction. It is also increasingly recognised that many cardiac drugs such as the angiotensin converting enzyme inhibitors and cardiac glycosides affect the autonomic nervous system and that these effects may be crucial to the overall therapeutic utility of these drugs (dioxin, ACEI). Non-invasive real time monitoring of cardiac vagal tone using the technique validated here promises to offer an important new tool for the cardiac physiologist and physician alike.

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Dog	Resting period	Mean CIPA (n=500)	Standard Deviation
1	Pre-Saline	40.5	9.6
	Pre-Atropine	21.5	9.7
2	Pre-Saline	58.9	14.1
	Pre-Atropine	49.8	11.7
3	Pre-Saline	12.3	7.1
	Pre-Atropine	21.5	9.6
4	Pre-Saline	68.7	21.7
	Pre-Atropine	50.4	14.7
5	Pre-Saline	47.3	15.8
	Pre-Atropine	63.5	14.2
6	Pre-Saline	25.3	6.8
	Pre-Atropine	32.3	10.3
7	Pre-Saline	33.5	12.2
	Pre-Atropine	53.0	19.7
8	Pre-Saline	23.1	14.3
	Pre-Atropine	24.3	24.0
9	Pre-Saline	41.8	18.1
	Pre-Atropine	42.4	13.7
10	Pre-Saline	38.0	8.7
	Pre-Atropine	78.9	14.2

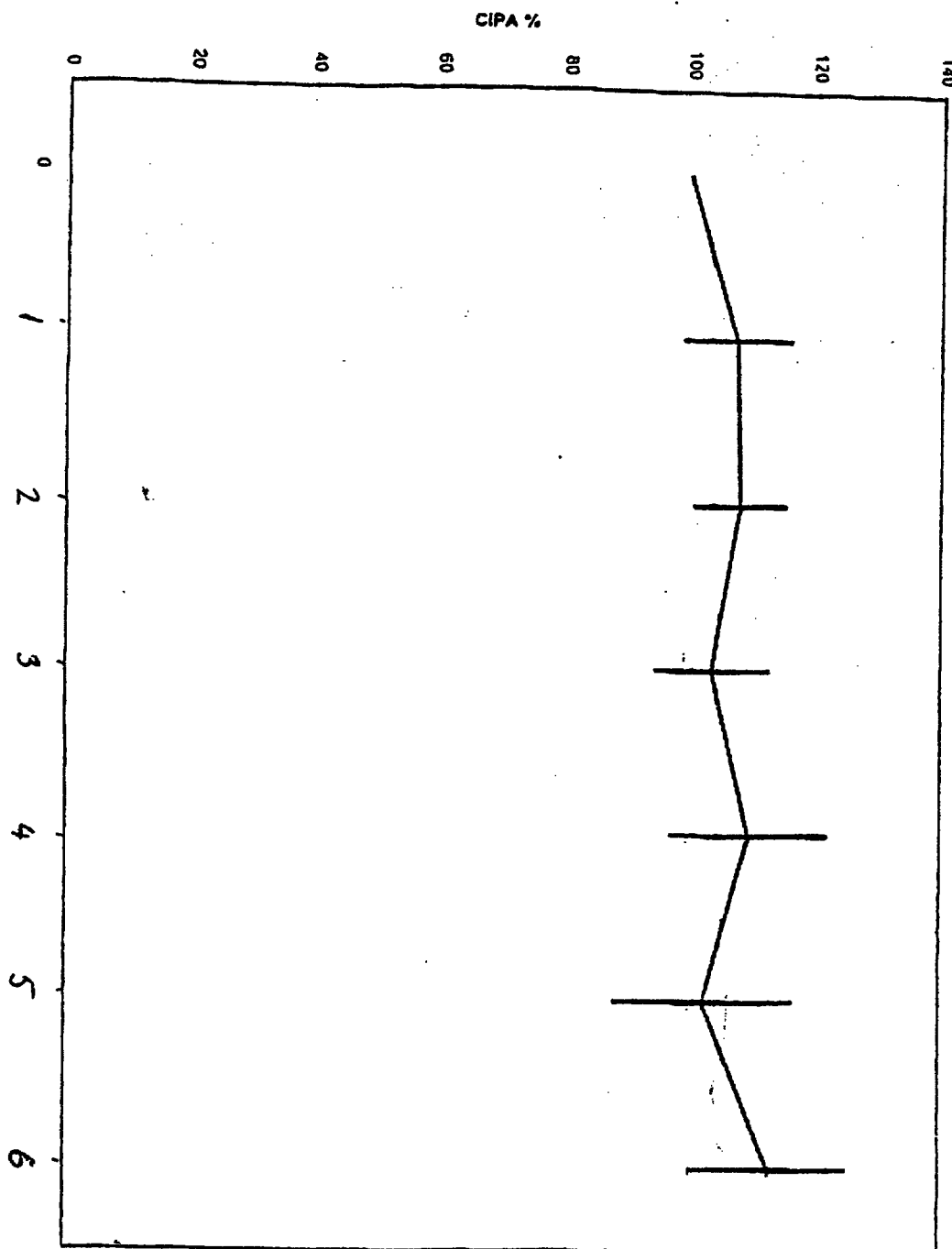


Fig. 1.

Saline Control

Effect of saline I.V. injection on CIPA values measured repeatedly in conscious dogs. Values are expressed as a percentage of the baseline value before injections began. Graph shows the mean and standard error of the mean for six consecutive injections of saline given at intervals of ten minutes to ten healthy experimental beagles. There are no significant differences between the CIPA values over this time frame.

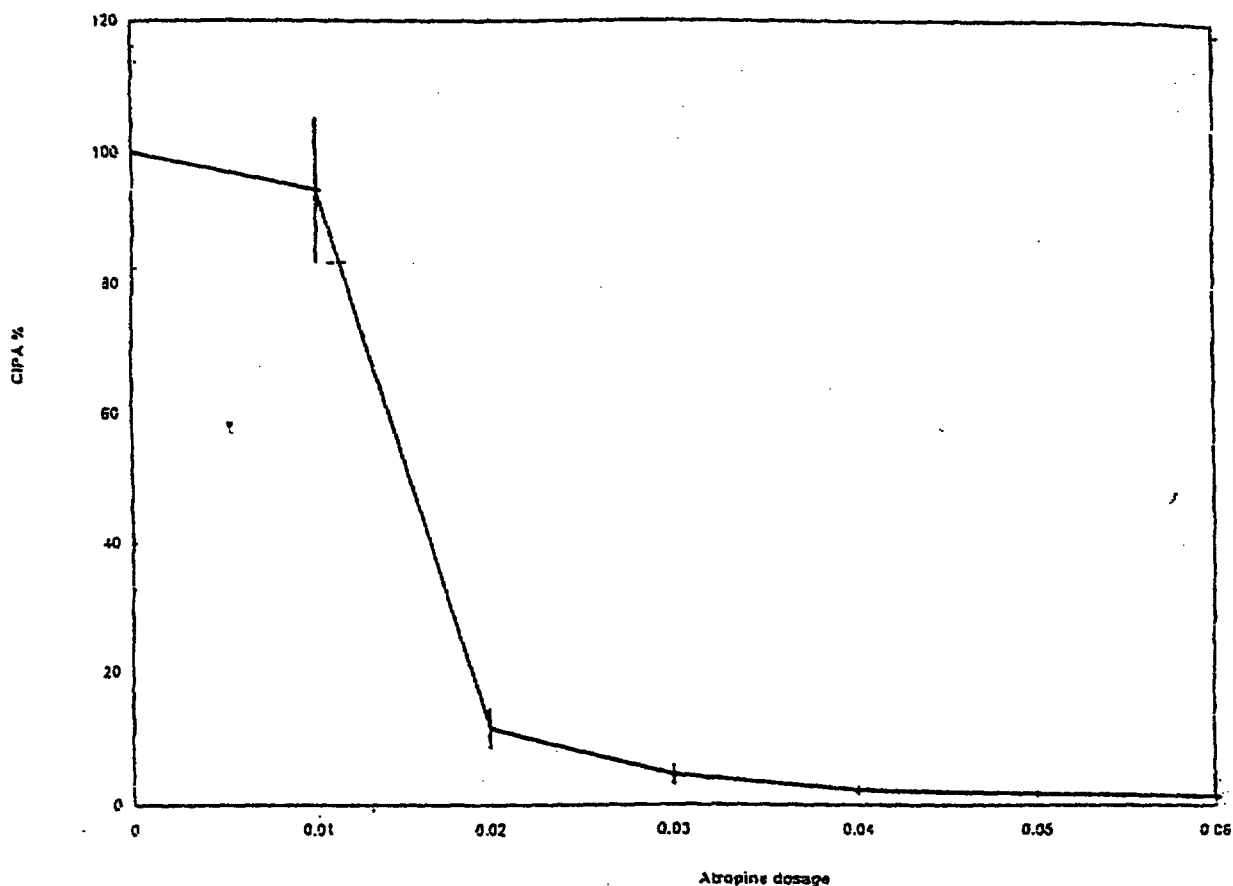


Fig. 2.

Effect of intravenous injection of atropine on CIPA values measured repeatedly in conscious dogs. Values are expressed as a percentage of the baseline value before injection commenced. Graph shows the mean and standard error of the mean for six consecutive injections of 0.01 mg/kg of atropine in saline given at intervals of ten minutes to a maximum dose of 0.06mg/kg to the same ten healthy beagles shown in Figure 1. There is a clear dose response relationship and at values of 0.04 mg/kg and above the CIPA index is all but obliterated in all dogs studied. Differences between dosages were statistically significant. Note that the SEM values fell dramatically as the mean CIPA values fell.

this index also fell dramatically when atropine was administered. In the control experiments, by contrast, a series of boluses of saline had no effect on the CIPA index in these dogs (Figure 1). Following atropinisation induction of the oculocardiac reflex by pressing on the eyes had no noticeable effect on the CIPA index or heart rate thus confirming that cardiac vagal tone had been obliterated. When saline was administered in place of atropine this reflex remained intact.

Atropinisation reduced the mean R-R wave interval in all dogs and after a cumulative dose of 0.06mg/kg this value had fallen to a mean of 51.5 percent (SD=11.5%, range 30.3 - 67.3%) of the value at baseline. Standard deviation of the R-R wave interval also fell in each of these dogs as atropinisation proceeded, but the residual value of this index was higher than the residual CIPA value in all dogs at the maximum atropine dosage (mean = 10.6%, range; 2.9 - 22.5% and SD; 6.5 as a percentage of the baseline value).

Statistical analysis of the CIPA data showed that there was a significant *dose x treatment* interaction term which confirmed the observations from Figure 2 that the response in the treatment groups differed at different doses. ANOVA applied to the normalised atropine group data alone demonstrated a significant difference between baseline (dose 0) and dose 1 (0.01mg/kg), between 1 and 2, and between 2 and 3 but not between the higher doses.

Second degree heart block was observed in all dogs at some point during the atropine response experiment usually after the second or third aliquot of atropine had been administered intravenously.

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Functional evidence of brain stem immaturity in Rett syndrome

Abstract Autonomic activity and respiration were studied in Rett syndrome (RS) and age matched controls. Breathing movements were monitored using a pletysmograph around the chest. Sympathetic activity was monitored by measuring blood pressure (BP) using the FinapresTM. Cardiac parasympathetic activity was monitored by measuring the cardiac response to baroreflex using the NeuroScope which outputs a measure of cardiac vagal tone (CVT) in units of a linear vagal scale (LVS). Resting CVT (means $\pm$ SEM) was 10.5 ± 0.9 units in the LVS and BP was 94.6 ± 6.4 mmHg in controls. The BP was 78 ± 4.33 mmHg and CVT was 3.6 ± 0.7 units in the LVS in girls with RS, 65% lower than in their age matched controls ($p < 0.001$), but equal to previously reported level in neonates. Each girl with RS had at least 6 types of breathing dysrhythmias, a sign of instability of the respiratory oscillator. The sympathetic system

controlled the HR and BP smoothly during breath holding in control girls, but there were oscillations and rebounds in RS. The HR and BP were under parasympathetic influence during hyperventilation in normal girls but not in RS. The CVT was invariably withdrawn at the height of sympathetic activity during both hyperventilation and breath holding in RS, leading to sympatho-vagal imbalance with the risk of cardiac arrhythmias and possibly sudden death. Neonatal level of CVT, poor autonomic integration and multiple breathing dysrhythmias shows medullary immaturity in RS. It is the first demonstration of immaturity of the brain which could be used for screening in early childhood and potentially useful for diagnosis and management of RS.

Key words Rett syndrome - autonomic - sympathetic - parasympathetic - respiration

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Introduction

So far the genetic flaw and biochemical defect in Rett Syndrome (RS) are unknown. Irregular breathing in which intense hyperventilation alternates with apnoea is common in the RS and is associated with extreme agitation, vacant spells and faints. Epileptogenic abnormalities are seen on the encephalogram and seizures are common. The girls give the impression of sympathetic

overstimulation with large pupils, flushed face and cold feet which are improved by sympathectomy.

Medullary respiratory and cardiovascular neurones are inseparable anatomically and are known to interact at synaptic levels to the extent that some workers regard them as cardiorespiratory neurones (11). It is indeed remarkable that there appears to be autonomic disturbances in association with the respiratory dysrhythmia in RS. Respiratory dysrhythmia is common in RS as shown in the British survey where families recog-

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nised hyperventilation in 75%, apnoeic attacks in 70%, non-seizure vacant spells in 77% and epileptic seizures in 70% of 191 classic cases. Another important and worrying feature of RS is sudden death. Of all reported deaths from RS in Britain, 25% were sudden and unexpected raising further questions about the integrity of the autonomic nervous system in the disorder. Abnormal autonomic function could underlie both the disturbed behaviour and the risk of sudden death in RS.

The group of cardiovascular neurones in the medulla oblongata perform autonomic functions that can be measured non-invasively. The work by Sun and Guyenet (14) establishes a linear relationship between the firing rate of a group of medullary sympathetic neurones in the rostral ventrolateral medulla and the mean arterial blood pressure in the range 80–160 mmHg. The rostral ventrolateral medulla is also known as the vasomotor "centre" or medullary cardiovascular "centre" responsible for the sympathetic tone in the peripheral vasculature. Monitoring the mean arterial blood pressure non-invasively would, therefore, constitute an indirect and non-invasive assessment of the sympathetic tone in the peripheral vessels. We can also measure cardiac vagal tone using the baroreflex mechanism which is the most important means of generating the tone. Pulse synchronised fast alterations of the cardiac periods are caused by the effects of the baroreflex on the sino-atrial node mediated through the nucleus ambiguus in the medulla and passed to the heart as vagal tone (3). This can be measured non-invasively from the ECG P-P intervals (3). The baroreflex mechanism, therefore, provides a noninvasive means of measuring the cardiac vagal tone generated from the nucleus ambiguus in the medulla oblongata.

We have used the above principles of noninvasive measurement of autonomic functions to monitor the autonomic activity in a group of girls with RS and their age matched controls, who were normal and healthy girls. The girls with RS had involuntary respiratory dysrhythmias such as breath holding and or hyperventilation during the period of our study. The healthy girls were, therefore, asked to perform voluntary breath holding, hyperventilation and deep breathing exercises during the study to illustrate the autonomic effects of such respiratory dysrhythmias in normal individuals of their age group.

Subjects, Material and Methods

Subjects

Seven girls with RS aged 4–14 years (means, $\pm$ SEM): 3.6 ± 1.4 years and nine normal and healthy girls aged 6–12 years 9 ± 0.6 years were recruited into the study with the approval of the local Ethics Committee. Other family members remained with the girls throughout the

study. The breathing movements, rate of breathing, cardiac vagal tone (CVT), cardiac response to baroreflex (CRB), beat-to-beat systolic, mean and diastolic blood pressures (BPs), and beat-to-beat heart rate (HR) were measured continuously during quiet rest in all the girls, during hyperventilation and immediately after it, during breath holding and deep breathing exercises in 5 of the girls with RS but all the control girls.

Breathing

Breathing movement was monitored using a stretch sensitive plethysmograph placed around the chest at the level of the Xiphisternum. Inspiratory and expiratory movements were recorded continuously into a computer. Inspiratory intervals were measured by accurate identification of the starts of consecutive inspirations. Breath-to-breath breathing rate was calculated from the inspiratory intervals to study the breathing rhythm and its relationships with autonomic function. Recording was continued until enough of the involuntary breathing dysrhythmias in girls with RS was obtained. The healthy control girls performed hyperventilation, breath holding and deep breathing under instructions.

Autonomic function

The methods of autonomic assessment are entirely painless and non-invasive and were developed for diagnostic purposes. A computerised, integrated monitoring system recorded beat-to-beat heart rate (HR) from the ECG R-R intervals, beat-to-beat systolic, mean and diastolic blood pressures (BPs) were measured non-invasively using the Finapres blood pressure monitor (Ohmeda, Eagleswood, USA). The Finapres photoplethysmographic cuff was applied on one finger from where continuous digital arterial BP wave forms was obtained for the calculation of the various beat-to-beat BPs. As sympathetic activity is closely related to the mean arterial BP (14), it was monitored indirectly, continuously and non-invasively using the mean BP reading from the Finapres™.

A continuous measure of cardiac vagal tone (CVT) was monitored in real time from the ECG signals using a modification of the principles described by Julu (5) and now implemented in the NeuroScope (Pontoppidan, Copenhagen, Denmark). To understand how we can quantify nerve impulses in the vagus nerve non-invasively, we must first appreciate that these impulses are integrated, or added up in the sino-atrial node and tend to affect the heart periods beat-by-beat. Work by Eckberg (3) showed that baroreceptor stimulation by neck suction had the maximum effect on the sino-atrial node (SA-node) when the stimulus was applied in synchrony

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with the ejection of blood into the great vessels indicated by the rise in arterial pressure. The most relevant part of the results of this study to measurement of vagal tone was that such stimulations caused a delay in the onset of the cardiac cycle immediately following the stimulus and this delay could be measured in the ECG P-P intervals (3). The delay in the onset of cardiac periods caused by baroreceptor stimulation is mediated through vagal tone (3). Since blood is ejected into the arteries in every cardiac cycle with enough strength to stimulate the baroreceptors, there is quantifiable pulse synchronised fast alterations in R-R intervals which is measured continuously by the NeuroScope. A scale of vagal tone known as the linear vagal scale (LVS) has been derived with the help of atropine for use with these fast alterations of R-R intervals (6). Since the LVS was obtained using graded doses of atropine, there is no further need of the use of atropine to prove that the NeuroScope actually measures cardiac parasympathetic activity each time the machine is used on patients. The dose-response curve to atropinization fitted the known pharmacophysiological relationships between cardiac vagal tone and atropine (6). The LVS will read 10 units in young adults in supine position and breathing normal tidal volumes quietly, and it will read zero units in every person at full atropinization (6).

If we define the cardiac baroreflex sensitivity, which is in fact the cardiac response to baroreflex (CRB), as the increase in pulse intervals per unit change in BP, we can measure the CRB by quantifying cardiac responses to ejection pressures in each cardiac cycle as $\Delta RR/\Delta SBP$, where ΔRR is the difference between present and previous ECG R-R intervals and ΔSBP is the difference between systolic pressures in two cycles preceding the current cardiac cycle. This method is based on the observation by Eckberg (3) in which a brisk stimulation of baroreceptors affected the period of the cardiac cycle immediately following the stimulus. Beat-to-beat ABPs are measured using FinapresTM, while the beat-to-beat ΔRR s are obtained from consecutive R-R intervals measured by the NeuroScope. These are then integrated into a single microcomputer which calculates the CRB (5).

Monitoring of respiration and autonomic functions was carried out continuously for up to two hours or until sufficient periods with quiet rest, hyperventilation and possibly breath holding have been recorded in girls with RS. The period of monitoring was shorter in healthy control girls who were asked to perform the breath holding, hyperventilation and deep breathing voluntarily after 30 min of quiet baseline records.

Data Presentation and Analysis

Mean values of CVT, mean BP, and respiratory rates measured during quiet rests in controls were compared

with those in girls with RS using unpaired Student's *t*-tests. The dynamic changes of autonomic functions during respiratory dysrhythmias were treated as real time graphs and some examples are given in the Results. Important patterns of changes in respiration or autonomic function and differences between controls and girls with RS are qualitatively described in the Results.

Results

Baseline medullary functions

Breathing rhythms

The normal breathing patterns in controls and girls with RS are shown in Fig. 1 in which every inspiration is invariably followed by expiration, often without delay. Such a normal breathing pattern was observed in every girl with RS, but the periods at which they were sustained uninterrupted by dysrhythmias varied considerably, lasting as short as 4 min in one girl or as long as 50 min in another within the 2 hour period of our recordings.

There were 9 different types of respiratory dysrhythmias observed in the 7 girls with RS (Fig. 2). Each girl had at least 6 different types of dysrhythmias with usually one dominant type coming more often than the rest. The common respiratory dysrhythmias in the 7 girls with RS were breath holding, central apnoea, rapid shallow breathing, hyperventilation, tachypnoea and deep breathing (Fig. 2). Bior's breathing (Fig. 2) which is periodic abrupt apnoea and equally abrupt regular breathing of equal depth in which both the apnoea and the regular breathing have variable lengths, was observed in only one girl with RS. Regular short breath holds (Fig. 2) were seen in three girls. Cheyne-Stokes breathing (Fig. 2) was observed in five girls and was either unprovoked or following hyperventilation or breath holding. Cheyne-Stokes breathing following a long breath holding has not been reported in normal

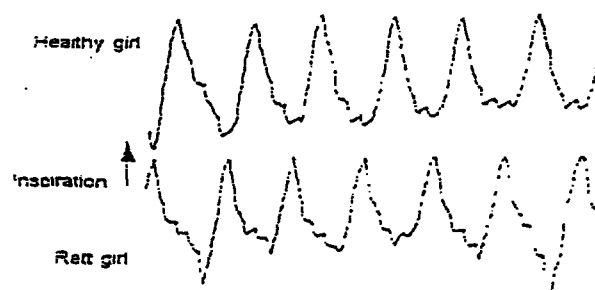


Fig. 1 Normal breathing pattern in a girl with Rett's syndrome and a healthy control. The depths of breathing are equal and inspirations are sharp and short, while expirations are nearly biphasic

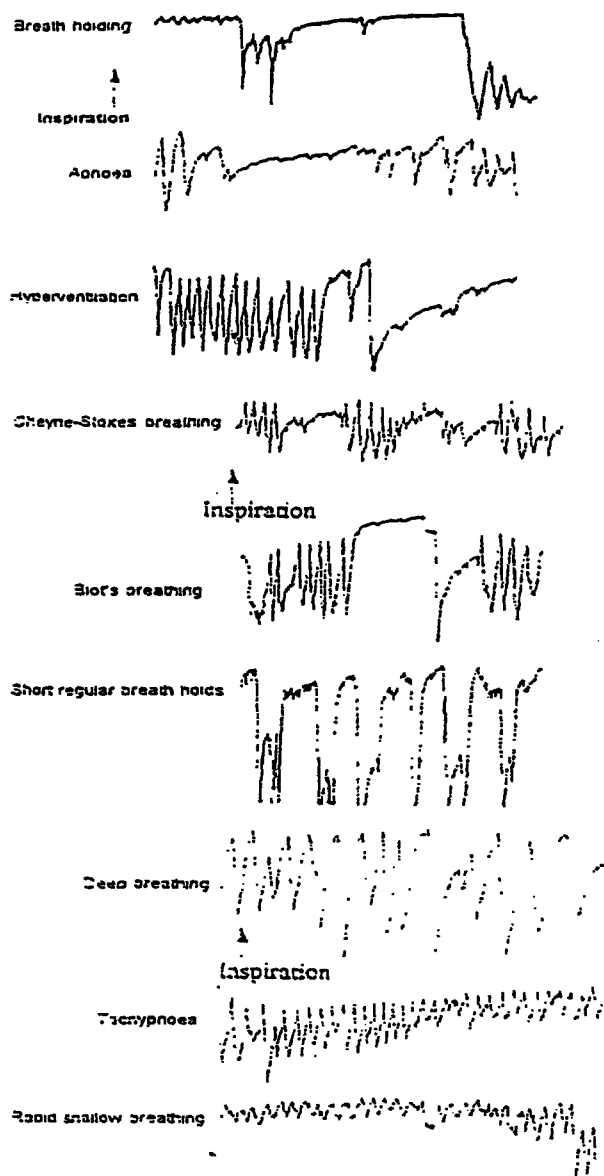


Fig. 2 Breathing dysrhythmias in Rett's syndrome. Breath holding were often ended with a sharp burst of expiration. Central apnoea tended to interrupt normal breathing, while hyperventilation was often followed by apnoea or Cheyne-Stokes breathing. Biot's breathing and short regular breath holds were rare. Deep breathing often followed long breath holds. Tachypnoea and rapid shallow breathing were interspersed among the other dysrhythmias.

subjects of this age group in the literature, but was observed here only in girls with RS and not in controls. These respiratory dysrhythmias were sustained for variable periods during our recordings, rarely exceeding 2 min before changing to another pattern.

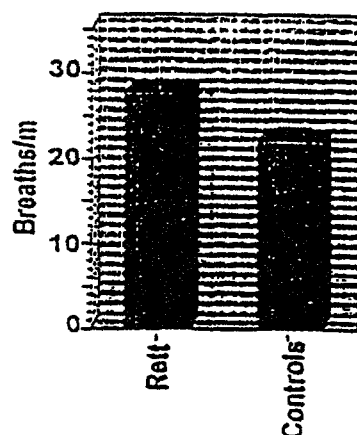


Fig. 3 Averages of normal breathing rates in girls Rett's syndrome and controls. There was no statistically significant difference between them ($p > 0.2$).

Breathing rate

Normal resting breathing rate varied between 18–28 breaths/min in controls and 16–34 breaths/min in girls with RS. The mean breathing rates in controls and in girls with RS measured during normal breathing patterns in quiet rests are given in Fig. 3. There was a trend towards higher breathing rates in girls with RS when compared with the age matched controls, but this was not statistically significant.

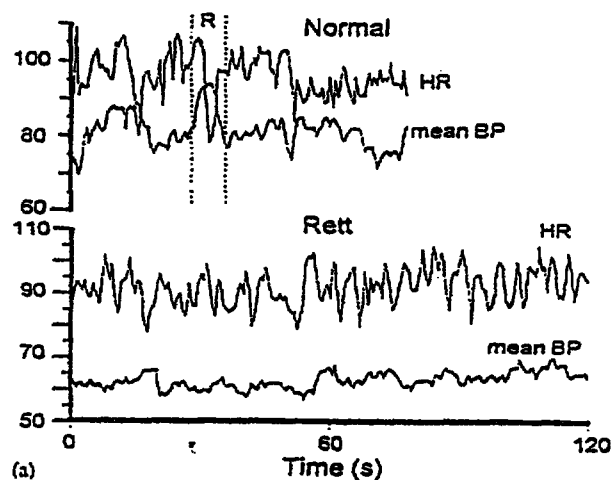
Autonomic function

Maintenance of mean arterial blood pressure (indirect assessment of sympathetic activity)

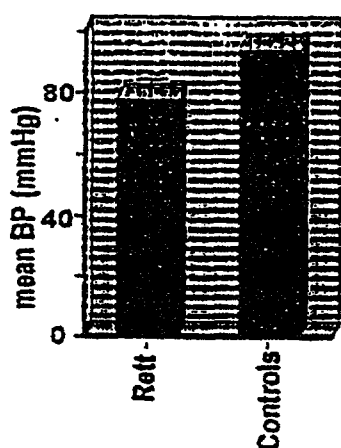
The mean arterial BPs measured continuously in a healthy girl and one with RS during quiet rest and normal breathing are shown in Fig. 4a to illustrate that the heart rate (HR) frequently responded to limit large variation in mean BP in the healthy girl but not in RS. The averages of mean BPs during quiet rest in all the controls and girls with RS are compared in Fig. 4b. The BP varied from 68–120 mmHg in controls and 60–90 mmHg in girls with RS. There was, thus, a trend towards lower resting mean BPs in girls with RS when compared with controls, but this was not statistically significant (Fig. 4b).

Resting cardiac vagal tone

Examples of the resting cardiac vagal tone (CVT) measured continuously in a healthy girl and one with RS in quiet rest during normal breathing are shown in Fig. 5a to illustrate that the resting CVT frequently responded



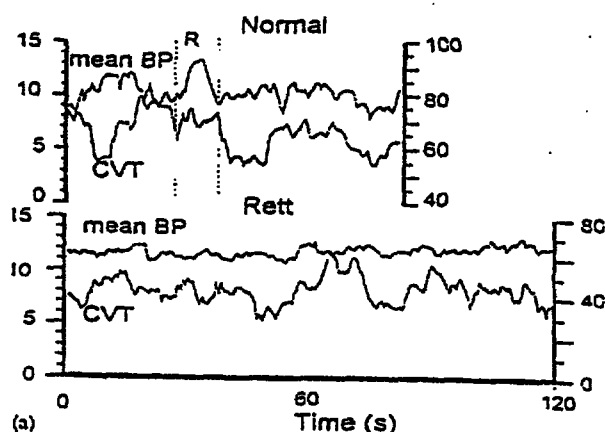
(a)



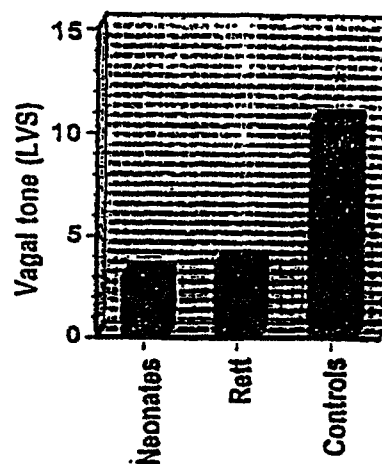
(b)

Fig. 4 (a) Continuous and simultaneous recording of heart rate (HR) and mean arterial blood pressure (mean BP) in a girl with Rett's syndrome (Rett) and a normal healthy girl (Normal) at rest and during normal breathing are shown. A sharp drop in HR at R within the vertical broken lines in the normal girl was in response to a brisk rise in BP which was successfully corrected back to the baseline level. There are no such responses in the Rett girl. The BP in the Rett girl remained below 70 mmHg while BP varied between 75–85 mmHg in the normal girl. Figure 4b compares the averages of BP in all Rett and normal girls. There was no statistically significant difference the two ($p>0.1$).

to limit large changes in mean arterial BP in the healthy girl but not in RS, just what the HR did in Fig. 4a. It could be inferred that the HR responded to the changes in CVT. Figure 5b compares the averages of CVT in all controls and girls with RS. There was a significantly large difference in the resting CVT in girls with RS when compared with their age matched controls, the CVT being 65% lower in RS than in controls ($p<0.001$).



(a)



(b)

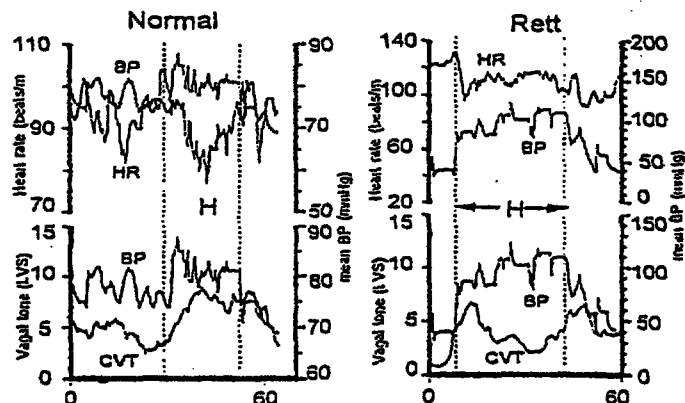
Fig. 5 (a) Continuous and simultaneous recording of cardiac vagal tone (CVT) and mean arterial blood pressure (mean BP) in a girl with Rett's syndrome (Rett) and a normal healthy girl (Normal) during normal breathing are shown. There was a sustained high level of CVT at R enclosed within the vertical broken lines in the normal girl in response to a sharp rise in BP, but no such responses in the Rett girl. Figure 5b compares the averages of the resting CVT in three groups of children. The level of CVT in the Rett girls was about equal to a previously reported neonatal level (4) and significantly lower than in controls ($*p<0.001$).

Integrative medullary cardiorespiratory functions

Autonomic response to hyperventilation

Figure 6 shows the responses of the HR, CVT, and mean arterial BP representing sympathetic activity to hyperventilation in a healthy girl in comparison to that of a girl with RS. The healthy girl was breathing quietly at 22 breaths/min and was able to switch immediately to 60 breaths/min when asked to hyperventilate. There was a sharp increase in mean BP which was immediately countered by a fall in HR in response to an

Fig. 6



increase in vagal tone (Fig. 6). This successfully controlled the blood pressure to within the basal level and once this was achieved, the HR was then restored to the basal level in a V-shaped manner (Fig. 6). Vagal tone remained elevated during the period of hyperventilation, but was withdrawn to baseline level when the girl stopped hyperventilating. This was the pattern of activity in all the normal girls during their voluntary hyperventilation. It was evident that the heart and indeed the cardiovascular system was under increased parasympathetic regulation during hyperventilation in these healthy girls.

The girl with RS was also breathing quietly at 20 breaths/min, a similar rate to that of the healthy girl, before she gradually and involuntarily started hyperventilating. Her respiratory rate increased from 20 to 60 breaths/min gradually in 15 s and it took nearly the same period for the breathing rate to decay to end the hyperventilation which was maintained for about 30 s in this girl. Vagal tone increased initially, but was withdrawn in the middle of hyperventilation to be reinstated during the decay of the hyperventilation (Fig. 6). There was a sharp increase in the mean BP at the beginning of the hyperventilation which could not be countered by a meagre fall in HR due to the early withdrawal of vagal tone. The BP was, therefore, uncontrolled during hyperventilation leading to a run-away increase in mean BP which rose from 40 mmHg to 110 mmHg, an increase of 70 mmHg. This was associated with agitation, distress and vocalisation in this girl. There were similar patterns of activity in four girls with RS who hyperventilated for at least 15 s or more without interruption during our recording. Three girls with RS hyperventilated for less than 10 s during our recording which was too brief for the study of cardiorespiratory integration.

The sympatho-vagal balance during hyperventilation in the two groups of girls was as follows: There was increased vagal tone to counter increased sympathetic activity indicated by raised BP in healthy control girls.

Raised vagal tone was maintained throughout hyperventilation and beyond in these healthy girls. While in the girls with RS, there was withdrawal of vagal tone at the height of sympathetic activity creating severe imbalance between the two cardiac autonomic tones, sympathetic and vagal, right in the middle of all the hyperventilation that lasted for more than 15 s without interruption.

Autonomic response to breath holding

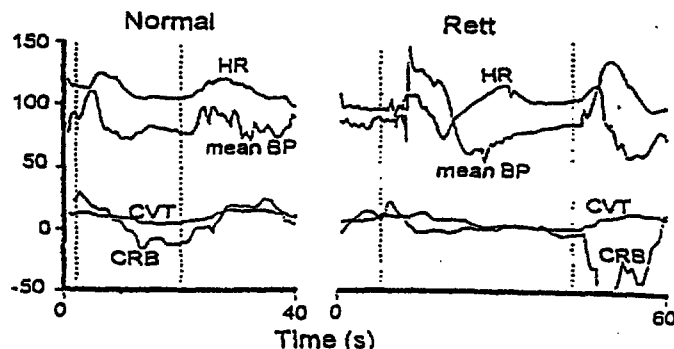
Figure 7 compares examples of the responses of the HR, CRB, CVT and mean arterial BP representing sympathetic activity in a normal girl with those in a girl with RS during breath holding. Healthy girls held their breath voluntarily for at least 20 s. Baroreflex control of the cardiovascular system was promptly and appropriately withdrawn, then restored immediately at the end of breath holding in all healthy girls in a similar manner to what is illustrated by one of them in the example in Fig. 7. The vagal tone was gently withdrawn and reinstated when breathing resumed. The HR and BP had similar patterns of responses throughout breath holding, an indication that they were under the same regulatory influence of the sympathetic system. It was evident that the whole cardiovascular system was under the influence of the sympathetic nervous system during voluntary breath holding, while the influences of vagal tone and baroreflex were withdrawn in the healthy girls. However, both the vagal tone and baroreflex influences were reinstated back in an orderly fashion when breathing was resumed in these healthy girls (Fig. 7).

Breath holding was involuntary in girls with RS. They held their breath for periods varying from 20 s to 90 s and in the example in Figure 7, the girl with RS held her breath for more than 30 s. Baroreflex regulation of the cardiovascular system was also withdrawn quickly and kept at or near zero throughout the breath holding similar to what was observed in healthy girls.

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Fig. 7 ■■■■



but CRB was not reinstated when breathing resumed. Vagal tone was withdrawn smoothly and was gently reinstated when breathing resumed like in healthy girls. The increase in sympathetic activity at the beginning of breath holding which could be observed in both HR and BP caused oscillation or "ringing" mainly in the BP, a sign of poor restraint of the sympathetic system by the negative feedback mechanism of the baroreflex known to control it (13). The recovery phase which came when breathing resumed was exaggerated as indicated by the response of the HR which increased above the baseline level in a rebounding manner. This is another indication of poor function of the negative feedback control mechanism of the sympathetic system. The rebound coincided with complete withdrawal of vagal tone leading to severe imbalance between sympathetic and vagal tones at the end of a prolonged breath holding in RS. The evidence provided by these results shows that the whole cardiovascular system was left under the control of the sympathetic nervous system during the involuntary breath holding in RS which is similar to what was seen during the voluntary manoeuvres in healthy girls. However, the sympathetic control of BP and HR in girls with RS was poor and predisposed to spurious and exaggerated activity such as "ringing" or oscillations and rebounds.

Discussion

We have used a novel method of measuring the function of medullary neurones and many of our observations can be explained by immaturity of the medulla oblongata in RS. The level of the resting CVT of 3.6 ± 0.7 units in the LVS in RS was not only 65% lower than in their healthy age group, but almost identical to the 3.0 ± 0.6 units in the LVS previously reported in awake and quiet neonates two to three days old (+). This would suggest that the resting CVT in RS did not progress with age, it remained stagnant at the neonatal level. Sympathetic control of the HR and BP during breath

holding in RS was predisposed to ringing and rebounds which is absent in healthy girls as it would be prevented by a negative feedback mechanism in the baroreflex (14). Thus the ringing and rebounds in RS indicate a lack of this negative feedback regulation of the sympathetic system probably due to immaturity of the neurones involved. The multiple respiratory dysrhythmias in each girl with RS suggests instability of the respiratory oscillator present in the medulla oblongata (10) probably also due to immaturity. Unprovoked Cheyne-Stokes breathing is well documented in neonates, but in older children and adults, Cheyne-Stokes breathing is usually secondary to known causes of reduced pO_2 or pCO_2 or both in the blood (17). Our results show that girls with RS do have unprovoked Cheyne-Stokes breathing similar to neonates, a further indication of immaturity of medulla oblongata. We observed Cheyne-Stokes breathing in healthy girls following at least 20 s of hyperventilation which we used as the physiological indicator of sufficient reduction in pCO_2 in the blood, enough to affect the central breathing rhythm.

This is the first unequivocal functional indication of immaturity of the Rett brain and more specifically in the brainstem where our non-invasive techniques have a great advantage over other existing methods of assessment of brain function. However, immaturity of other areas of the Rett brain has been suggested by neuropathological reports of specific reductions in dendritic territories in the motor, frontal and inferior temporal cortex without evidence of progressive degenerative change (1, 2). Single Proton Emission Computed Tomography of the Rett brain showed patterns similar to those in neonates, indicative of the immature pattern of brain activity in the Rett brain (9). All these provide a consistent evidence to suggest that RS is a developmental disorder characterised by neuronal immaturity or hypoplasia affecting largely the integrative function of the brain. Our results suggest that agitation and some of the associated vocalisation in RS could be the result of uncontrolled sympathetic activity arising from poor autonomic integration. This is consistent with the character-

istic wide pupils, cold extremity and flushed face in RS, which are all implications that the lack of autonomic integration could be a fundamental abnormality in the RS.

Integrative function of medullary respiratory neurones has been studied extensively and may give us some idea of what could be wrong in RS. At least three groups of medullary neurones interact in a co-ordinated fashion to generate a stable respiratory rhythm (10). Over or under activity of any one group of these neurones can cause respiratory dysrhythmia of one sort or the other (10). All the girls with RS in this present study were able to generate and maintain a normal breathing pattern to some extent. It means the respiratory oscillator in RS is capable of generating a normal breathing pattern. However, the activity of the groups of neurones serving the function of the respiratory oscillator is modulated by a number of other neurones using different types of neurotransmitters such as opioids (8), serotonin (7), purines (12) and others. These modulatory neurones could probably be immature or abnormal in RS causing instability in the breathing pattern. Sero-

tonergic neurones are known to modulate the activity of expiratory neurones and they also prevent apnoea (7). Lack of the modulating effect of serotonergic neurones could therefore lead to frequent apnoea which can be interpreted clinically as breath holding and other types of the respiratory dysrhythmias we have observed in RS could ensue. For this reason, the integrity of serotonergic neurones in the brainstem of girls with RS needs to be investigated in future studies.

The sympatho-vagal imbalance we observed during prolonged hyperventilation and breath holding in RS can cause cardiac arrhythmia and sudden death. Such imbalance between vagal and sympathetic tones in the heart has been reported as the cause of sudden deaths in post-ischaemic myocardial infarction (16, 18), and it has also been suggested as a probable cause of sudden death in acute insulin induced hypoglycaemia (15). Since at least 25% of all reported deaths in RS are sudden and unexpected, it would be reasonable to recommend a research into the prevention of prolonged hyperventilation or breath holding in RS by pharmacological means.

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Brief Communications

REFERENCE 2

Selective defect of baroreflex blood pressure buffering with intact cardioinhibition in a woman with familial aniridia

Article abstract—We report a symptomatic failure of the baroreceptor blood pressure (BP) buffering mechanism in a woman with familial aniridia. Her baseline BP oscillated at 0.1 Hz, the frequency of Mayer waves, with increased amplitude on standing without orthostatic hypotension. Although sudomotor function was normal, cutaneous thermoregulatory function and BP response to Valsalva's maneuver were abnormal. The defective BP buffer mechanism suggests Mayer waves could be a sympathetic mediated cardiovascular resonance. Baroreceptor cardioinhibition was intact. We presume that the lesion is in the rostral aspect of the dorsal medulla oblongata.

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Buffer nerves from the carotid and aortic sinuses carry the baroreceptor afferents used in the medulla oblongata for the generation of cardioinhibition and the reflex inhibition of the sympathetic system.¹ This constitutes a negative feedback regulatory mechanism of blood pressure (BP) referred to as BP buffering. The rationale of the clinical practice of carotid massage assumes that if cardioinhibition can be elicited in the procedure, the whole baroreflex mechanism is passed for normal. Our patient illustrates that the reflex inhibition of the sympathetic system can be defective with an intact cardioinhibition in the baroreflex mechanism. A possible explanation of the origin of Mayer waves is advanced from the observations in this patient.

Case report. A 32-year-old woman with familial aniridia had a 20-year history of episodes of light-headedness, nausea, and mild vertiginous symptoms. During two of the events she lost consciousness, with convulsive movements on one occasion, but no history of diabetes.

Vision was poor (6/60 bilaterally), with pendular nystagmus. The neurologic examination was otherwise normal. BP was unstable, without demonstrable postural hypotension. ECG and echocardiography were normal, as were MRI of the brain and EEG. Because of the BP variation and history of aniridia, autonomic assessment was requested. The patient was on no medication that would have affected autonomic function.

Autonomic examination. The following autonomic functions were assessed: Cardiovascular reflexes and cardiac parasympathetic function. Resting cardiac parasympathetic activity (CPA) was measured from the analogue ECG using the NeuroScope (Pontoppidan, Copenhagen, Denmark) in our autonomic laboratory. The NeuroScope quantifies the baroreflex-induced heart rate (HR) fluctuations in units of a linear vagal scale that have been validated using atropine.² Further tests included massaging the carotid sinus, a 10-second cycle of deep breathing, and an IV injection of phenylephrine (200 µg, bolus dose). Arterial BP was recorded continuously using the Finapres (Ohmeda, Eagleswood.), allowing us to measure the car-

diac sensitivity to baroreflex in real time,³ defined as the change in consecutive R-R intervals per unit change in beat-to-beat systolic BP (SBP).

Cardiac response to orthostasis was quantified using the 30:15 ratio test. The longest R-R interval occurring at around the 30th cardiac cycle following a data marker indicating an upright stance was divided by the shortest R-R interval occurring at around the 15th cardiac cycle after the marker to obtain the 30:15 ratio.

Response of arterial BP to orthostasis was assessed continuously using the Finapres. Systolic, mean arterial (MAP), and diastolic (DBP) BPs were measured in the digital artery of one finger. Readings of BP within 1 to 2 minutes of a change in posture are important for the assessment of the neural control of BP.⁴ Variation of MAP by more than 25 mm Hg suggested orthostatic instability, whereas a sustained fall in DBP by 10 mm Hg or more in the erect stance compared with supine position indicated orthostatic hypotension.

Responses of R-R intervals and BP to a pressor effect of isometric contraction of the muscles of the dominant forearm were defined as cardiac baroreflex responsiveness (CBR). This is the change in pulse interval per unit change in BP. It is the absolute value of the quantity $|R-R/BP_{\text{Systolic}}|$ where R-R is the change in R-R interval during isometric exercise in association with a corresponding change in SBP (BP_{Systolic}) from baseline levels.⁵

BP response to Valsalva's maneuver was assessed while the patient maintained active intrathoracic pressure of 40 mm Hg for 15 seconds. The four phases of BP response and the Valsalva's ratio in a continuous recording were used.

Sympathetic functions. Emotional sudomotor function (ESF) was assessed from the sympathetic skin response in the palms of the hands and soles of the feet evoked by a single inspiratory gasp. Responses were recorded at a sweep speed of 1 sec/division, sensitivity of 5 mV/division, high pass filter at 20 Hz, and low pass filter at 0.01 Hz.

Thermoregulatory vasomotor function (TVF) was assessed using a four-channel laser Doppler flow meter. Baseline skin blood flow was measured in each limb for 3 minutes, then a cold challenge was applied to one hand while recording the skin blood flow on the contralateral limbs. The challenge involved 2-minute immersions of the

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Table/Results of the assessment of the patient's cardiovascular reflexes compared with normal references in the laboratory

Cardiovascular reflexes	Patient	References
Baseline CPA (units of the LVS)	8.1	5-10
Baseline HR (beats/min)	71	60-80
Carotid massage (BP drop mm Hg)	17	10-25
Carotid massage (CPA in LVS)	5.2	5-20
CBR (msec/mm Hg)	2.78	2.07
Phenylephrine slope (msec/mm Hg)	21.0	6-14
Valsalva's ratio	2.39	1.21-1.80
30:15 ratio	1.60	1.10-1.30

Reference values are from 250 control subjects aged 13 to 79 years.

CPA = cardiac parasympathetic activity; HR = heart rate; BP = blood pressure; LVS = linear vagal scale; CBR = cardiac baroreflex responsiveness in isometric exercise; 30:15 ratio = ratio of the 30th to the 15th ECG R-R intervals following standing up-right from a low seated position.

hand in warm water (40 °C), then in cold water (10 °C), and finally back into warm water. Normal responses were indicated by decreased contralateral blood flow during cold challenge, and a return to baseline levels in response to re-warming. Failure of TVF was indicated by either no effect of cold challenge or a paradoxical increase in blood flow.

Change in HR in the third minute of isometric exercise was used to assess cardioaccelerator function, and the change in DBP was used to assess sympathetic tone. Increase in HR measured continuously in our laboratory is closely related to the change in DBP, proving the sympathetic origin of this cardioacceleration.² Moreover, by the third minute of isometric exercise, vagal and baroreflex regulation of the cardiovascular system would have been withdrawn.³

Results. Results of autonomic function tests. Resting rate of breathing was 17 breaths/min. The table shows the results of cardiovascular reflexes.

IV injection of phenylephrine increased SBP by 20 mm Hg associated with bradycardia, implying intact cardioinhibition (figure 1). Changes in beat-to-beat HR reciprocated the CPA, suggesting a causal relationship (see figure 1).

Sympathetic functions. DBP increased by 30.7 mm Hg during isometric exercise (normal range, 19 to 48 mm Hg). HR increased by 25% of baseline during the same exercise (normal range, 23 to 45%). The response of BP to Valsalva's maneuver was consistent with a reduction in venous return. Of the two cutaneous sympathetic functions tested, there was normal ESF while TVF was absent in all four limbs.

Stability of blood pressure. BP stability was so poor that it was oscillating at 0.1 Hz throughout the recording (figure 2). The amplitude of oscillation increased when the patient stood up, but the frequency was unchanged. The oscillations were so obvious that Fourier transform and subsequent power spectral analysis were not necessary for the elucidation.

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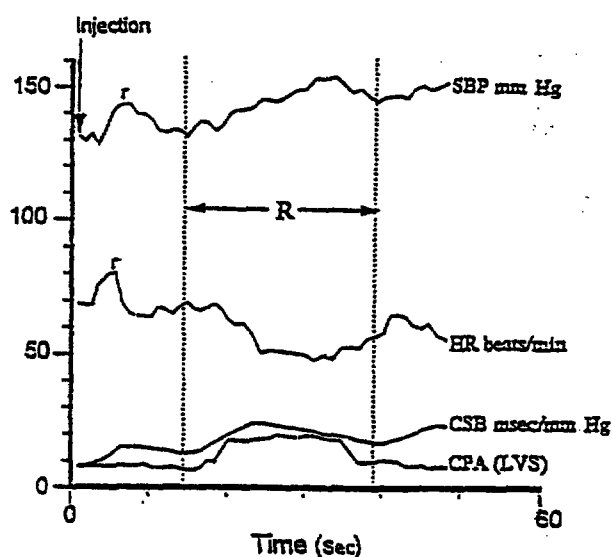


Figure 1. Continuous records of systolic blood pressure (SBP), heart rate (HR), cardiac sensitivity to baroreflex (CSB), and cardiac parasympathetic activity (CPA) expressed as a linear vagal scale (LVS). Phenylephrine injection at time zero (vertical arrow) caused a response in all the above parameters during the period R after about 15 seconds latency. There was an early, exclusively sympathetic, response without change in CPA at *t*, which was caused by the injection stimulus. A ramp increase in SBP in the period R caused bradycardia, which reciprocated the rise in CPA, a sign of an intact baroreflex cardioinhibition. The CSB increased during the period R.

Discussion. We describe a patient with abnormal baroreflex function indicated by the exaggerated Valsalva's ratio, abnormally large 30:15 ratio, and a phenylephrine slope greater than the normal range observed in our laboratory. These were superimposed on a persistent oscillation of BP and HR. However, the CBR in isometric exercise, the CPA increase, and BP drop caused by digital pressure on the carotid sinus were normal.

There are three main frequency bands in the beat-to-beat variation of BP and HR if studied by power spectral analysis in the time domain.⁶ The high-frequency peak is attributed to respiratory activity, but the causes of mid- (0.07 to 0.14 Hz) and low- (0.02 to 0.06 Hz) frequency variations are controversial.⁸ A mathematical model of neural regulation of the cardiovascular system predicts a sympathetic resonance phenomenon at 0.1 Hz,⁷ also known as Mayer waves.⁸ This mid-frequency cardiovascular variation has been attributed to baroreflex activity and proposed as an index of baroreflex sensitivity.⁸ The cardiovascular oscillation in our patient is consistent with a sympathetic resonance as predicted by the DeBoer model because its amplitude increased in the upright position when sympathetic activity is expected to increase. Because baroreflex activity diminishes on standing, it is unlikely to have caused

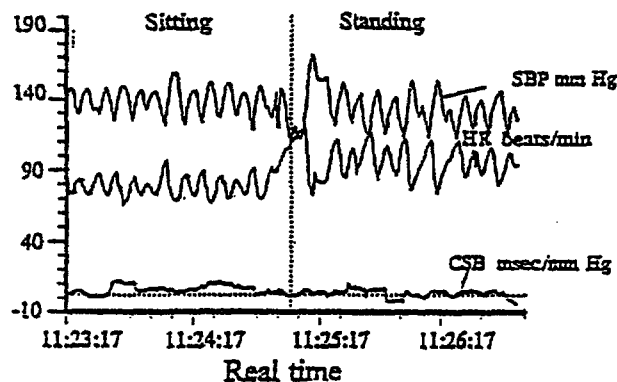


Figure 2. Continuous records of systolic blood pressure (SBP), heart rate (HR), and cardiac sensitivity to baroreflex (CSB) in sitting, during transition, and in standing postures. CSB was very low and remained so throughout the three postures. There was a steady oscillation of SBP and HR with a period of 10 seconds (0.1 Hz) during both sitting and standing postures. The phase of SBP oscillation was inversely related to that of the HR. Amplitude of SBP oscillation was 20 to 40 mm Hg in the sitting posture and increased to 40 to 70 mm Hg on standing. Normal BP drop and tachycardia during transitional posture was intact.

the oscillation. Baroreflex cardioinhibition was intact in our patient, as indicated by bradycardia caused by phenylephrine.

The extensive autonomic investigation of our patient allows us to speculate on the site of the lesion. Because it is largely the baroreflex BP buffering that is defective and cardioinhibition is intact, both of which are executed by afferents from the baroreceptors, we presume that the lesion may be in the nucleus of the tractus solitarius in the rostral part of the dorsal medulla oblongata. Secondary neurons diverge here. One group goes to the nucleus ambiguus to effect cardioinhibition, and another group goes to the sympathetic neurons in the rostral ventrolateral medulla to effect a reflex inhibition of the neurons. The rostral medulla would be a reasonable site for such target-specific lesions of the sympathetic sys-

tem illustrated in our patient by the selective cutaneous vasomotor failure. Spatial arrangement of sympathetic neurons in this part of the medulla is target specific.⁹ The lesion in our patient was undetectable by conventional brain imaging. This patient has given us a very rare opportunity to confirm a sympathetic origin of 0.1 Hz oscillation in the cardiovascular system, the so-called Mayer waves.

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CLAIMS

1. Apparatus for providing a measure of vagal tone in a human or animal patient and comprising:

a) input means for receiving an ECG signal obtained from a patient;

5 b) detection means coupled to said input for detecting QRS waveforms in said ECG signal and for generating timing data indicative of the periods between consecutively received QRS waveforms; and

10 c) frequency demodulation means coupled to said detection means for demodulating said timing data substantially in real time and for generating a signal corresponding to the frequency modulating signal in said ECG signal, wherein said generated signal is used to provide said measure of vagal tone.

15 2. Apparatus as claimed in claim 1 wherein the detection means is arranged to compare received portions of the ECG signal against a stored expected QRS waveform (or portion thereof), to identify when a QRS complex is received.

20 3. Apparatus as claimed in claim 1 or claim 2 wherein said timing signal comprises a sequence of fixed length pulses which are generated upon receipt of a QRS waveform.

25 4. Apparatus as claimed in any one of claims 1 to 3 wherein the frequency demodulation means is a frequency discriminator comprising a differentiator in series with an envelope detector.

30 5. Apparatus as claimed in any one of claims 1 to 3 wherein the frequency demodulation means is a phase locked loop (PLL) incorporating a voltage controlled oscillator (VCO) substantially tuned to the nominal constant heart rate.

35 6. Apparatus as claimed in claim 5 wherein the nominal rate or frequency of the VCO is preset or is determined by sweeping the VCO frequency across a suitable range until a lock is obtained with the nominal heart rate

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frequency of the received ECG signal.

7. Apparatus as claimed in any preceding claim wherein said frequency demodulation means comprises an integrator coupled to the detection means for receiving said fixed length pulses and a high pass filter and a low pass filter coupled separately to the integrator for respectively receiving an output of the integrator, the output of the high pass filter being coupled to a first voltage controlled oscillator via a second integrator and the output of the low pass filter being coupled to a second voltage controlled oscillator, the outputs of the said and first second voltage controlled oscillators being coupled to respective inputs of a phase comparator arranged to generate at its output a signal indicative of the phase difference between the two input signals.

8. Apparatus as claimed in claim 7 wherein said first and second integrators have a time constant between 1.0 and 2.5 seconds so that the outputs of the first and second voltage controlled oscillators are substantially in phase.

9. Apparatus as claimed in claim 7 or claim 8 wherein the output from the phase comparator is coupled to a third integrator, which has a time constant selected from the range 1.0 to 2.5 seconds.

10. Apparatus as claimed in claim 9 wherein a pre-set DC bias is added to the output of the third integrator to ensure that the resulting summed signal always exceeds zero.

11. Apparatus as claimed in any preceding claim including a filter coupled to the frequency demodulation means for filtering the frequency modulating signal to remove noise and other unwanted signal components.

12. Apparatus as claimed in any preceding claim wherein the apparatus includes signal processing means for converting the modulating signal into a linear vagal scale (LVS).

13. Apparatus for measuring vagal tone from an ECG

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according to the above first aspect of the present invention in combination with ECG recording means, said apparatus having an input means coupled to the output of the ECG recording means.

14. Apparatus as claimed in claim 13 wherein the ECG recording means includes electrodes for attachment to the patient and amplifiers for pre-amplifying the recorded ECG to feed an amplified signal to said input means.

15. A method for providing a measure of vagal tone from an ECG recorded from a human or animal patient, the method comprising the steps of:

detecting the occurrence of QRS waveforms in the ECG signal;

generating a timing signal indicative of the time period between consecutively received QRS waveforms;

frequency demodulating said timing signal in real time to obtain a frequency modulation signal, wherein said modulation signal is used to provide said measure of vagal tone.

16. A method as claimed in claim 15 wherein the method comprises the further step of filtering the ECG, the timing signal, or the modulation signal to remove low frequency components.

17. A method as claimed in claim 16 wherein the frequency demodulating step comprises integrating the generated pulse sequence, and separately high pass and low pass filtering in parallel paths the integrated signal, said high pass filtered signal then being integrated and the resulting integrated signal used to frequency modulate a first carrier signal, and said low pass filtered signal being separately used to frequency modulate a second carrier signal, the phase differences between the first and second modulated carrier signals being used to generate an output signal proportional to the phase differences between said input signals.

18. Apparatus as claimed in any one of claims 1 to 14 for use in diagnosing spongiform encephalopathies in a

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human or animal patient.

19. Apparatus as claimed in any one of claims 1 to 14 for use in diagnosing bovine spongiform encephalopathy.

5 20. A method of facilitating diagnosis of bovine spongiform (BSE) encephalopathy in cattle, the method comprising;

measuring the vagal tone of a subject cow using the method of the above third aspect of the present invention;

10 comparing the measured vagal tone against the vagal tone expected for a healthy cow; and

providing in indication of the difference between the two compared tones to assist in the diagnosing of BSE.

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