REGULATION OF ANAESTHESIA

The present invention relates to the regulation of anaesthesia and also a method of evaluating the anaesthetic needs of a subject.

The metabolic activity of the brain changes in various clinical situations. For example the metabolic activity of the brain is increased during an epileptic fit and during rapid eye movement sleep. In contrast the metabolic activity of the brain is reduced during hibernation and during the administration of a general anaesthetic.

Anaesthesia may be defined as a loss of feeling or insensibility to external stimuli. Anaesthesia may be local (the loss of sensation in a specific tissue) or general (when it is generally associated with a lack of consciousness). Studies have shown that a reduction in brain metabolism of some 47% is associated with a state of general anaesthesia. Administration of excessive doses of anaesthetic compounds leads to a reduction in metabolic activity in excess of this level and a depth of anaesthesia that is excessive and associated with an increased risk of side-effects. It is therefore particularly important for a clinician to be able to reliably and sensitively regulate brain activity to allow the induction of controlled anaesthesia.

A state of anaesthesia is physiologically different to sleep. For instance, a subject who is asleep may be easily roused and therefore remains sensitive to external stimuli whereas a subject under a general anaesthetic may not be roused to consciousness by external stimuli. Furthermore sleep is not necessarily associated with reduced brain activity (e.g. during Rapid Eye Movement sleep, brain activity is normally high) whereas general anaesthetic is associated with reduced activity. Given the differences between anaesthesia and sleep it is not surprising that anaesthetic compounds do not necessarily act as hypnotics and *vice versa.*

Small, volatile molecules which induce anaesthesia (e.g. alcohols, halothane, ether etc) have been known for many years and are, or have been, commonly used to

induce and maintain anaesthesia prior to, and during, elective surgery etc. However many conventional anaesthetics have various disadvantages. These include:

(1) narrow concentration range over which the agent is effective (too little and the subject regains sensitivity to external stimuli whereas too much results in coma or death);

(2) slow recovery following anaesthesia;

(3) common side effects such as respiratory depression, cardiovascular instability and vomiting; and

(4) uncommon but life threatening side-effects such as malignant hyperpyrexia.

Therefore there is a need to provide compounds which may be used as, or with anaesthetics, which obviate or mitigate disadvantages associated with the prior art.

According to a first aspect of the present invention, there is provided the use of a compound which modulates Delta-Sleep Inducing Peptide activity for the manufacture of a medicament for regulating anaesthesia.

DSIP is a nonapeptide (which can exist in linear or cyclic form) with the amino acid sequence:

Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu

DSIP was discovered in the 1970's and has been proposed for sleep induction (for which it has had only limited success) and for treating drug addicts during drug withdrawal. However it has not previously been associated with anaesthesia and we have found that compounds which modulate DSIP activity are able to regulate anaesthesia.

DSIP may cause a reduction in brain metabolism which may be associated with a changed level of consciousness. However, the inventors have established that the reduction in brain metabolism seen with anaesthesia leads to a change in consciousness which is not typical of normal sleep. In fact, following DSIP treatment

there is a decrease in the amount of Rapid Eye Movement sleep and an increase in delta wave activity. The inventors have correlated these changes with the anaesthetised state and have therefore established that compounds which modulate DSIP activity may be used according to the first aspect of the invention. The inventors further believe that DSIP may be important in the induction of hibernation and the reduction of brain metabolic activity during hibernation and similar states.

The inventors believe that DSIP is an endogenous "anaesthetic-like" substance which modulates neurotransmission and brain activity. This belief is founded upon observations made whilst conducting studies using PET to assess metabolic activity changes that occur in various areas of the brain during anaesthesia with conventional anaesthetic agents. The invention arose from the realisation that the areas of the brain in which there were changes in metabolic activity in response to a conventional anaesthetic agent were the same areas where DSIP has been shown to be located using immunohistology techniques.

Although we do not wish to be bound by any hypothesis, we believe that compounds which modulate DSIP activity are effective because they regulate binding of ligands with a neuromodulatory binding site on neuroreceptors which have been linked to the regulation of anaesthesia (e.g. the site described by Mihic *et al.* (1997) Nature 389 p385-389 on $GABA_A$ receptors and glycine receptors). We believe binding of DSIP to these receptors modulates signalling from these receptors and thereby regulates the level of brain metabolism and the level of anaesthesia.

Our hypothesis that DSIP acts as an anaesthetic was confirmed by experiments which established that administration of DSIP induces anaesthesia and also prolongs anaesthesia induced by other anaesthetic agents. For instance, anaesthesia following a 7mg/kg iv bolus of propofol was approximately 28% longer in animals pretreated with DSIP (1mg/kg IP, 15 mins prior to the propofol bolus) compared to animals treated with propofol alone. Further experimental data illustrating the efficacy of DSIP, and related compounds, is presented in the Example below.

According to a first embodiment of the first aspect of the invention, we have found that compounds which increase DSIP activity may be administered alone, or preferably in combination with certain other anaesthetic agents, to induce or maintain anaesthesia. When used as part of a regime to induce anaesthesia, compounds which increase DSIP activity may be administered at the time of induction or at an earlier time as part of a regimen of pre-medication.

Several classes of compound which are capable of increasing DSIP activity may be used according to the invention. Such compounds include agonists or partial agonists of DSIP neuromodulatory binding sites, agents which enhance the release of endogenous agonists of DSIP neuromodulatory binding sites, agents which enhance the synthesis of endogenous agonists of DSIP neuromodulatory binding sites, agents which attenuate the breakdown (or removal/sequestration) of endogenous DSIP agonists, agents which increase DSIP expression or activity and agents which enhance the mechanisms involved in signal transduction between the ligand bound DSIP binding site and effector systems.

Preferred compounds which increase DSIP activity are DSIP agonists and include DSIP *per se* and derivatives and/or pharamaceutically acceptable salts thereof.

Preferred DSIP agonists which may be used according to the first embodiment of the first aspect of the invention include the phosphorylated nonapeptides disclosed in British Patent No. 2 000 511. (which are incorporated herein by reference).

Biologically active fragments of DSIP, biologically active DSIP derivatives and larger peptides comprising the nonapeptide (or biologically active fragments and derivatives thereof) are also preferred compounds for use according to the first embodiment of the first aspect of the invention. For example a preferred derivative of DSIP is Cyclo(-GLY-DSIP) which is described by Nekrasov *et al.* (Biochem. Mol. Biol. Int. 1996:38 p739-745). This derivative is more lipophilic than DSIP and crosses the blood brain barrier more readily. Cyclo (-GLY-DSIP) is particularly useful for rapid induction of anaesthesia.

It will be appreciated that non-peptide compounds which mimic peptide DSIP agonist activity (which may be isolated from nature or rationally designed) may also be used.

Compounds which modulate DSIP activity may be used in a method of inducing anaesthesia comprising administering to a patient to be anaesthetised an effective amount of a compound which promotes DSIP activity to induce at least part of the desired level of anaesthesia.

We believe that DSIP (and functional analogues thereof) induce or maintain anaesthesia according to the first embodiment of the first aspect of the invention for the following reasons:

(1) It is a neuromodulator, not necessarily a neurotransmitter, which we believe influences a transmembrane binding site on the $GABA_A$, glycine and possibly other receptors in a manner consistent with a modulator working via the same site as the ethanol site and/or the enflurane anaesthetic site.

(2) It is an anticonvulsant.

(3) It has analgesic properties. We believe DSIP acts as an analgesic because it promotes the release of met-enkephalin.

(4) Studies to investigate a possible action of DSIP in sleep promotion have shown that it does not induce normal sleep stages but promotes delta wave activity on the electroencephalograph as do many anaesthetics. During anaesthesia the electroencephalograph shows a complex pattern which may include a delta wave component but this pattern is distinct from that seen during natural sleep stages.

(5) It may regulate excitation and inhibition within the brain. It may modulate thermoregulation, as do general anaesthetics.

The analgesic properties of the compounds (3 above) represents a particular advantage of compounds used according to the first embodiment of the first aspect of the invention. Under certain circumstances the analgesic activity of a compound may outlast the anaesthetic action. This is of particular benefit as it will promote pain relief

during a recovery period following surgery etc. Furthermore it will be appreciated that the analegesia promoted by the compounds is not associated with respiratory depression (a common side-effect of many known analgesics e.g. morphine).

The inventors have found that compounds which increase DSIP activity are particularly useful for treating patients who require long term ventilation in the intensive care setting. A problem associated with such patients relates to the long term maintenance of an adequate state of anaesthesia such that the patient is maintained pain free and can be ventilated. Extensive clinical experience has shown that increasing doses of anaesthetic agents are required. In general, gaseous agents are not used because of a number of major drawbacks including pollution of the local environment. Continuous intravenous anaesthesia using propofol is often used. However, accumulation of elements of the propofol formulation results in undesirable effects. Another major problem is that tolerance to the anaesthetic effects of propofol develops, in some cases rapidly, such that ever larger doses are required to maintain the patient. Finally, when the time comes to wean patients off the anaesthetic in order to wean the patient off the ventilator, the respiratory depression caused by conventional general anaesthetics is a major problem. The use of compounds that increase DSIP activity in this clinical setting has particular advantages because increased DSIP activity does not cause respiratory depression. Furthermore tolerance has not been observed to the effects of the naturally occurring hormone. In addition, DSIP has activities that will confer additional benefits over many conventional anaesthetics as follows:

1) DSIP has been shown to have analgesic activity of its own, possibly through the release of met enkephalin; (pain is frequently a prominent problem in the long term ventilated patient); and
2) DSIP has been shown to have a beneficial effect on the adaptive responses to stress (the intensive care setting is extremely stressful).

We have found that compounds which increase DSIP activity are also particularly useful as adjuncts to other anaesthetics. When given in conjunction with other anaesthetics, compounds that increase DSIP activity prolong the duration of

anaesthesia. Equally, when a compound according to the first embodiment of the first aspect of invention is used as an adjunct, a satisfactory depth of anaesthesia may be achieved at a reduced level of the other anaesthetic (compared to use of other anaesthetics alone). This has the advantage of reducing the risk of side effects and/or the discomfort associated with recovery from the use of higher amounts of anaesthetic compounds. For instance, known anaesthetics can be associated with respiratory depression whereby patients stop spontaneous breathing. DSIP is not associated with respiratory depression. Therefore, administration of DSIP with a reduced level of known anaesthetic results in an acceptable level of anaesthesia without respiratory depression.

The use of DSIP and other compounds according to the first embodiment of the first aspect of the invention has the advantage that there is less risk of cardiovascular instability. Other advantages of using the compounds include:

(i) the kinetics of DSIP *in vivo* is non-saturable (metabolism is by plasma and other non-specific esterases);

(ii) peptide compounds such as DSIP are not toxic and need not be used as a gas. Therefore there is less environmental pollution during manufacture, use and disposal; and

(iii) compounds which promote DSIP activity also allow for instantaneous reversal, or at least quicker reversal, of general anaesthesia thereby further improving or eliminating anaesthetic recovery times and improving anaesthetic safety (e.g. the use of DSIP as an anaesthetic cofactor in combination with propofol helps smooth out propofol induced anaesthesia and allows fewer intraoperative side effects)

DSIP is degraded by a number of non-specific peptidases including Angiotensin Converting Enzyme (ACE). Therefore it is preferred for some applications that compounds according to the first embodiment of the first aspect of the invention are formulated with (or co-administered with) ACE inhibitors in order that DSIP activity may be potentiated. This is preferred when DSIP needs to be used for relatively long periods of time (e.g. anaesthesia and analgesia during intensive care).

According to a second embodiment of the first aspect of the invention compounds may be used which decrease DSIP activity.

Compounds according to the second embodiment of the first aspect of the invention may be used for increasing brain activity for inducing recovery from anaesthesia.

Several classes of compound which are capable of decreasing DSIP activity may be used according to the second embodiment of the first aspect of the invention. Such compounds include antagonists or partial agonists of DSIP neuromodulatory binding sites, agents which inhibit the release of endogenous agonists of DSIP neuromodulatory binding sites, agents which inhibit the synthesis of endogenous agonists of DSIP neuromodulatory binding sites, agents which promote the breakdown (or removal/sequestration) of endogenous DSIP agonists, agents which decrease DSIP expression or activity and agents which inhibit the mechanisms involved in signal transduction between the ligand bound DSIP binding site and effector systems.

Preferred compounds which decrease DSIP activity are DSIP anatagonists and include melatonin, dalargin and neokyotorphin.

A preferred use of compounds which decrease DSIP activity is to promote recovery from anaesthesia. Thus, immediately before an operation, compounds according to the first embodiment of the first aspect of the invention may be used (alone or in conjunction with another anaesthetic) to anaesthetise a subject and then, once the procedure has been completed, compounds according to the second embodiment of the first aspect of the invention may be used to expedite recovery from anaesthesia.

Brain activity may be regulated with compounds which modulate DSIP activity according to either embodiment of the first aspect of the invention as a

monotherapy or in combination with other agents. For instance, anaesthesia may be induced with compounds according to the first embodiment of the first aspect of the invention alone (a monotherapy) or in combination with other known anaesthetic agents (e.g. combination therapy with a DSIP agonist as an anaesthetic cofactor for propofol or with a gaseous agent to reduce MAC. MAC being the Minimum Alveolar Concentration of anaesthesia necessary to achieve loss of movement to a noxious stimulus in 50% of subjects).

When the compounds are used in combination with other agents, a lower dose of that agent may be required. This will reduce the incidence and severity of side-effects known to be caused by such agents. The dose requirements are typically reduced by 20 – 50% depending upon the specific combination used.

The compounds used according to the first aspect of the invention may take a number of different forms depending, in particular on the manner in which the composition is to be used. Thus, for example, the composition may be in the form of a powder, tablet, capsule, liquid, ointment, cream, gel, hydrogel, aerosol, spray, micelle, liposome or any other suitable form that may be administered to a person or animal. It will be appreciated that the vehicle of the composition of the invention should be one which is well tolerated by the subject to whom it is given and enables delivery of the compounds to the target tissue.

Preferred formulations include sterile, isotonic solutions for injection and micronised powders with excipients for oral inhalation.

The compounds may be used in a number of ways. For instance, systemic administration may be required in which case the compound may be contained within a composition which may for example be administered by injection into the blood stream. Injections may be intravenous (bolus or infusion) or subcutaneous (bolus or infusion). The compounds may also so be administered by inhalation. Alternatively the compound may be ingested orally in the form of a tablet, capsule or liquid.

Compounds modulating DSIP activity may be administered centrally by means of intracerebral, intracerebroventricular, or intrathecal delivery.

It will be appreciated that the amount of a compound required is determined by biological activity and bioavailability which in turn depends on the mode of administration, the physicochemical properties of the compound employed and whether the compound is being used as a monotherapy or in a combined therapy. The frequency and/or rate of administration will also be influenced by the above mentioned factors and particularly the half-life of the compound within the subject being treated. It will be appreciated that an anaesthetist will need to monitor the depth of anaesthesia of a subject during anaesthesia and adjust the required dose of the compound as required.

Known procedures, such as those conventionally employed by the pharmaceutical industry (e.g. *in vivo* experimentation, clinical trials etc), may be used to establish specific formulations of compositions and precise therapeutic regimes.

Generally, a dose of between 0.01 μg/kg of body weight and 1.0 g/kg of body weight of a compound which modulates DSIP activity may be used for the regulation of brain activity depending upon which specific compound is used and the reason for regulating activity. For instance, a suitable dose of a DSIP agonist will be in the range of between 1.0 μg/kg and 1.0 mg/kg (preferably 20 - 400μg/kg). Purely by way of example a suitable dose of DSIP for use in combination with propofol (e.g. 7mg/kg I.V. bolus) for inducing anaesthesia is between 0.01mg and 100 mg/kg and preferably between 0.02 mg/kg and 10 mg/kg.

Administration may be required frequently or continuously depending upon the requirements of an anaesthetist. By way of example between 1μg/kg/hr and 1g/kg/hr, and preferably between 10μg/kg/hr and 100mg/kg/hr of DSIP may be required to maintain anaesthesia.

According to a second aspect of the present invention, there is provided a method of regulating anaesthesia comprising administering to a subject in need of treatment a compound which modulates Delta-Sleep Inducing Peptide activity.

The abovementioned compounds which modulate DSIP activity according to the first aspect of the invention may be used according to the method of the second aspect of the invention.

According to a third aspect of the present invention there is provided a method of evaluating the anaesthetic needs of a subject to be anaesthetised comprising assaying a sample taken from the subject for the presence of Delta-Sleep Inducing Peptide.

By "anaesthetic needs" we mean an assessment of the dose of an anaesthetic required to induce or maintain a desired level of anaesthesia.

We have found that anaesthetic dose requirements are directly related to endogenous levels of DSIP. Thus a pre-operative assay of DSIP levels in a subject (e.g. a simple urine or blood test screening for DSIP) provides an anaesthetic dosage guide for predicting anaesthetic requirements. Higher than average endogenous levels of Delta-Sleep Inducing Peptide assayed from the sample indicate the subject will have lower than average anaesthetic requirements. Lower than average endogenous levels of Delta-Sleep Inducing Peptide assayed from the sample indicate the subject will have higher than average anaesthetic requirements.

It will be appreciated that the normal range for endogenous DSIP will depend upon the assay employed and the population studied. Purely by way of example DSIP levels may be assessed using the assay described by Seifritz *et al.* (Peptides 1995; 16 (8); p1475 – 1481). Using this assay the range of DSIP in blood is approximately 0.1 – 11 ng/ml. Therefore subjects with DSIP levels greater than about 5.0 ng/ml are likely to need less anaesthetic than normal whereas subjects with DSIP levels less than about 5.0 ng/ml are likely to require more anaesthetic than normal.

A suitable assay for measuring DSIP levels in a sample is a quantitative immunoassay utilising antibodies raised against DSIP. For instance, the enzyme immunoassay described by Kato *et al.* (Neuroendocrinology 1984;39:p39-44) may be adapted for use as a pre-operative test to evaluate anaesthetic requirements. An alternative assay which may be used according to the third aspect of the invention is a radioimmunoassay (e.g. as described by Seifritz *et al.* Supra). It is preferred that the assay mediates a colourmetric change which may be interpreted by eye or spectrophotometrically.

The sample is most suitably a blood or urine sample.

Such a method may be used pre-operatively to evaluate the anaesthetic needs of elective surgical patients.

According to one embodiment of the third aspect of the invention, an anaesthetist, nurse or theatre technician may test a blood or urine sample from a subject a short while (approximately 30 minutes or less) before anaesthesia to evaluate the anaesthetic needs of the subject. This test may be by means of inserting into the sample a dip-stick which undergoes a colour change (depending upon the DSIP levels in the sample). An anaesthetist can then interpret the measured levels and adapt the anaesthetic regime accordingly.

The invention will be further illustrated by the following non-limiting Example.

EXAMPLE

Experiments were performed in rodents to evaluate the effect of DSIP on anaesthesia induced by propofol.

Methods

Nine female Sprague-Dawley rats weighing 230 to 287g had free access to water and rat Purina chow. All animals were maintained, cared for, and handled in accordance with IACUC animal utilization policy. Animals were divided into two groups to test the interactions between DSIP and the intravenous anaesthetic agent propofol (n=5) or the inhalational anaesthetic agent isoflurane (n=4). For the propofol test, rats were randomly selected to receive either Delta-sleep inducing peptide (Peninsula Labs, CA) 1 mg/kg i.p. in 3 ml of sterile water or just 3 ml of sterile water i.p. alone (placebo) 15 minutes prior to injection of propofol (Trademarks: Diprivan or Rapinoivet) 7 mg/kg i.v. into a tail vein over approximately 10 s. Following injection of propofol animals were tested for loss of righting reflex. On loss of righting reflex the animals were placed on their sides in the center of a large plastic bowl with a flat bottom. Sleep time was recorded as the time taken to regain righting with all 4 feet on the ground. The following week, those animals that had received DSIP now received placebo pretreatment and those that had received placebo now received DSIP pretreatment. Again sleep time was assessed for each rat, after giving each rat the identical dose of propofol that it had been given the previous week.

For the inhalational test, rats were placed on a rotating rod in the middle of an anaesthetizing chamber. The level of inhalational agent was slowly titrated upwards in 0.05% increments every 10-15 min until the rats could no longer walk forward on the rotating rod. At week one, rats were randomly selected to receive either DSIP 0.1 mg/kg i.p. 15 min prior to testing, or placebo. The following week rats were crossed over to the other treatment arm (i.e. placebo to DSIP and DSIP to placebo).

Data were analyzed with a paired two-tailed t-tests.

Results

Intraperitoneal injection of 1mg/kg DSIP did not cause any rat to loose consciousness. Rats did, however, display a paucity of movement almost immediately after i.p. injection of DSIP. The animals did appear to be under the influence of some pharmacologic effect following DSIP pretreatment, perhaps best described by noting that the rats appeared to have a "vacant" look about them when left undisturbed. The animals would, however, move appropriately when approached, but then would quickly resume a crouched position when left alone.

Sleep times following propofol iv injection (7mg/kg) for each animal are shown in Table 1.

Table 1

animal	Sleep time (Sec) DSIP 1mg/kg	Sleep time (Sec) placebo
1	406	242
2	527	446
3	748	577
4	637	581
5	737	689

Each animal slept longer when pretreated with DSIP. The mean sleep time for propofol alone was 477 +/- 158 Sec. The mean sleep time for DSIP pretreatment followed by propofol was 611 +/- 145. This difference was significant at the $P<0.01$ level and represents a mean 28% increase in sleep time.

The dose of isoflurane anaesthesia (chamber IS0%) required to prevent each animal from being able to walk forward on a rotating rod is shown in Table 2.

Table 2

animal	Chamber iso % DSIP 0.1mg/kg ip	Chamber iso % placebo
1	0.24	0.30
2	0.12	0.20
3	0.26	0.31
4	0.18	0.21

The mean (+/- SD) concentration of isoflurane that prevented animals from being able to walk on the rotarod following placebo alone was 0.26 +/- 0.06%. The DSIP pretreatment reduced this value 23% to 0.20 +/- 0.06%. This reduction was statistically significant at the $p = 0.01$ level.

These data illustrate that DSIP was particularly effective when used as an adjunct to both propofol and isoflurane. Table 1 illustrates that DSIP prolongs the length of anaesthesia whereas Table 2 illustrates that DSIP is able to lower the concentration of another anaesthetic which is required to induce anaesthesia.



CLAIMS

1. The use of a compound which modulates Delta-Sleep Inducing Peptide activity for the manufacture of a medicament for regulating anaesthesia.

2. The use according to claim 1, wherein the compound promotes or mimics Delta-Sleep Inducing Peptide activity and the medicament promotes or induces anaesthesia.

3. The use according to claim 2 wherein the compound is for use in conjunction with another anaesthetic agent.

4. The use of a compound which promotes Delta-Sleep Inducing Peptide activity for the manufacture of a medicament for promoting or inducing sedation.

5. The use according to any one of claims 2 - 4, wherein the compound is Delta-Sleep Inducing Peptide or biologically active fragments and derivatives thereof.

6. The use according to claim 5 wherein the compound is a nonapeptide with the amino acid sequence:

Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu

- or biologically active fragments and derivatives thereof.

7. The use according to claim 6 wherein at least one amino acid or derivative thereof is phosphorylated.

8. The use according to claim 1, wherein the compound inhibits Delta-Sleep Inducing Peptide activity and the medicament promotes or induces recovery from anaesthesia.

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9. A method of regulating anaesthesia comprising administering to a subject in need of treatment a compound which modulates Delta-Sleep Inducing Peptide activity.

10. A method of evaluating the anaesthetic needs of a subject to be anaesthetised comprising assaying a sample taken from the subject for the presence of Delta-Sleep Inducing Peptide.

11. The method according to claim 10, wherein the sample is a blood or urine sample.

12. The method according to claim 10 or 11, wherein higher than average endogenous levels of Delta-Sleep Inducing Peptide assayed from the sample indicate the subject will have lower than average anaesthetic requirements.

13. The method according to claim 10 or 11, wherein lower than average endogenous levels of Delta-Sleep Inducing Peptide assayed from the sample indicate the subject will have higher than average anaesthetic requirements.

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