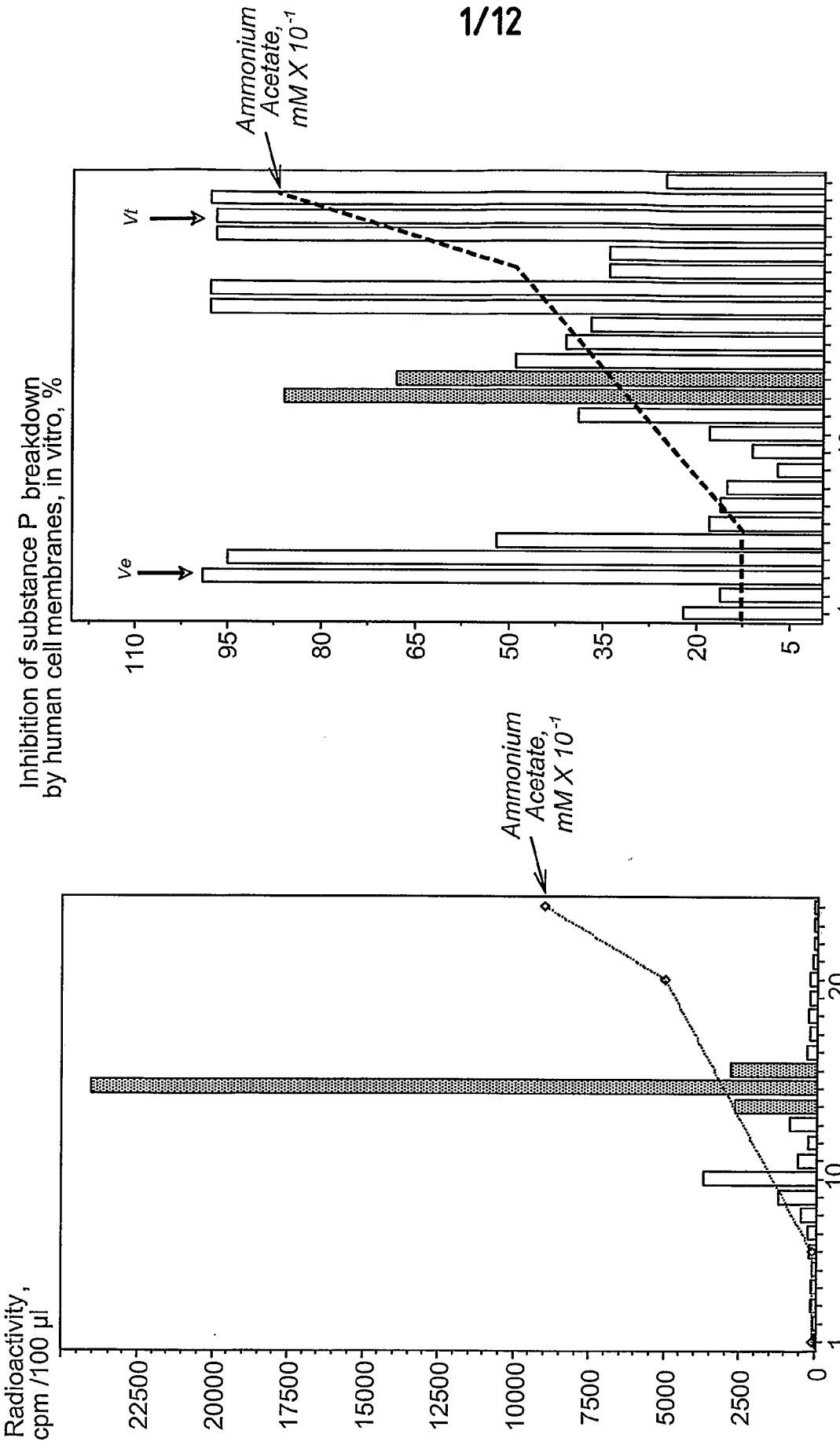

Radioactivity, cpm /100 µl
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
Ammonium Acetate, mM X 10-1
1
10
20
Fractions, 2 min/2 ml

FIG.1


Inhibition of substance P breakdown by human cell membranes, in vitro, %
110
95
80
65
50
35
20
5
Ve
Vt
Ammonium Acetate, mM X 10-1
1
10
20
Fractions, 2 min/2 ml

FIG.2

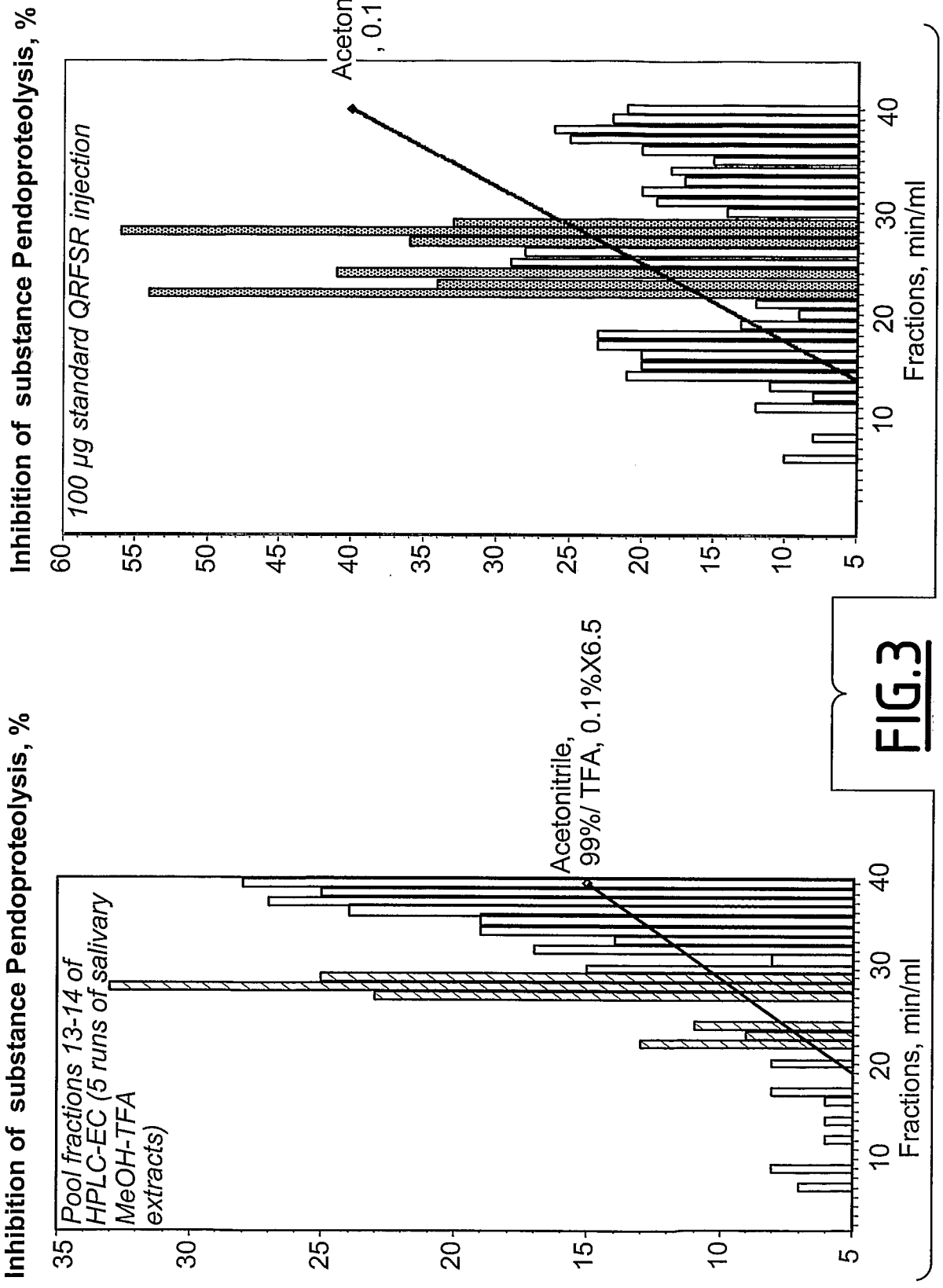

Inhibition of substance Pendoproteolysis, %
100 µg standard QRFSR injection
Acetonitrile, 99%/ TFA, , 0.1%X2.5
Fractions, min/ml
Inhibition of substance Pendoproteolysis, %
Pool fractions 13-14 of HPLC-EC (5 runs of salivary MeOH-TFA extracts)
Acetonitrile, 99%/ TFA, 0.1%X6.5
Fractions, min/ml

FIG.3

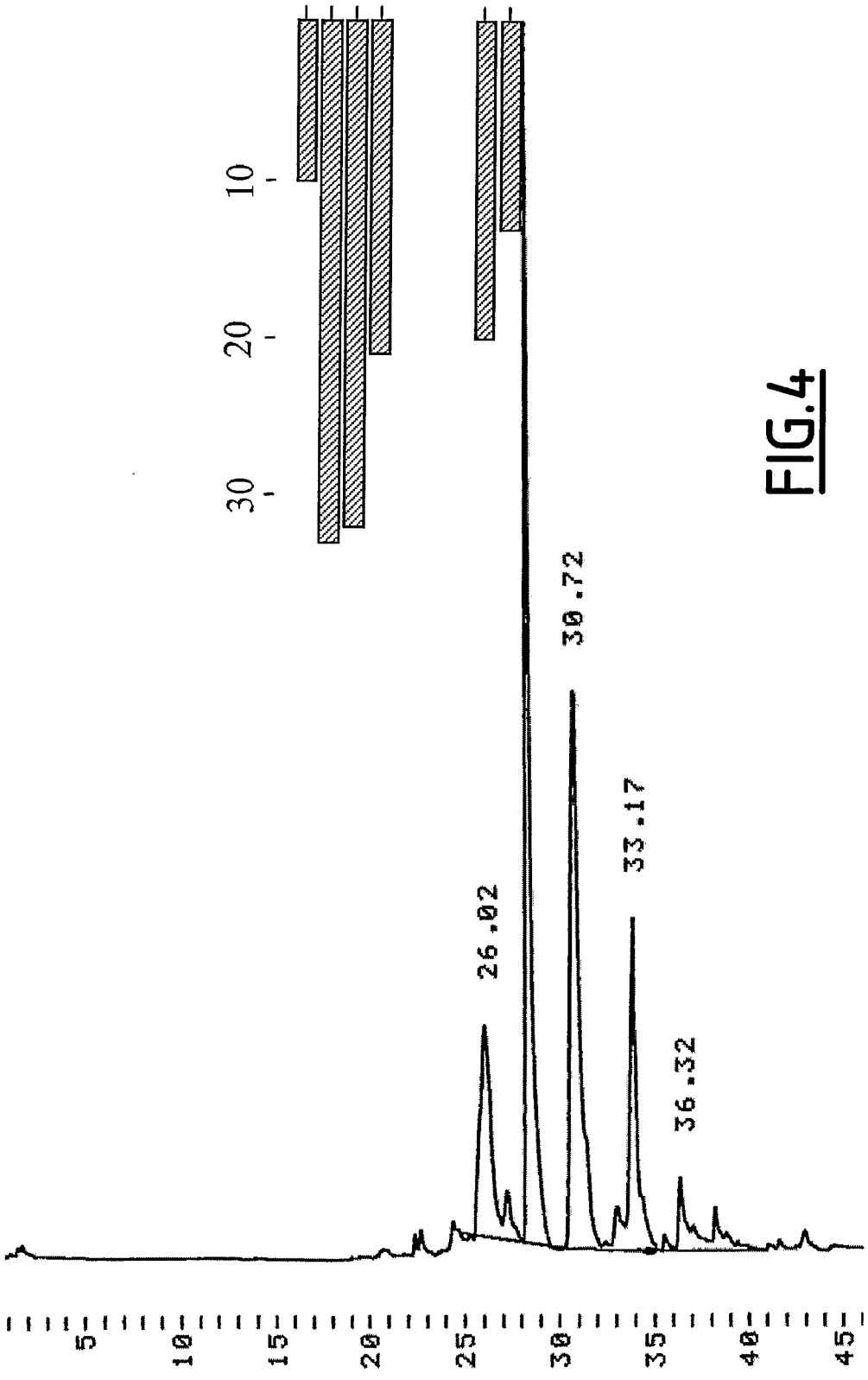



FIG.4

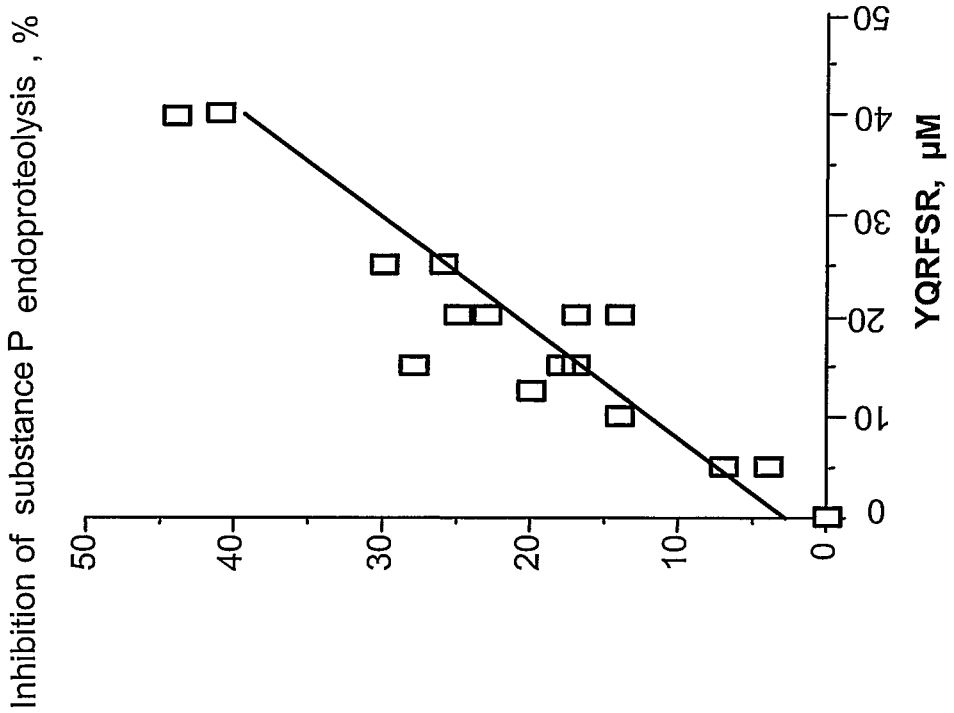

Inhibition of substance P endoproteolysis , %
YQRFSR, µM
0
10
20
30
40
50

FIG.6

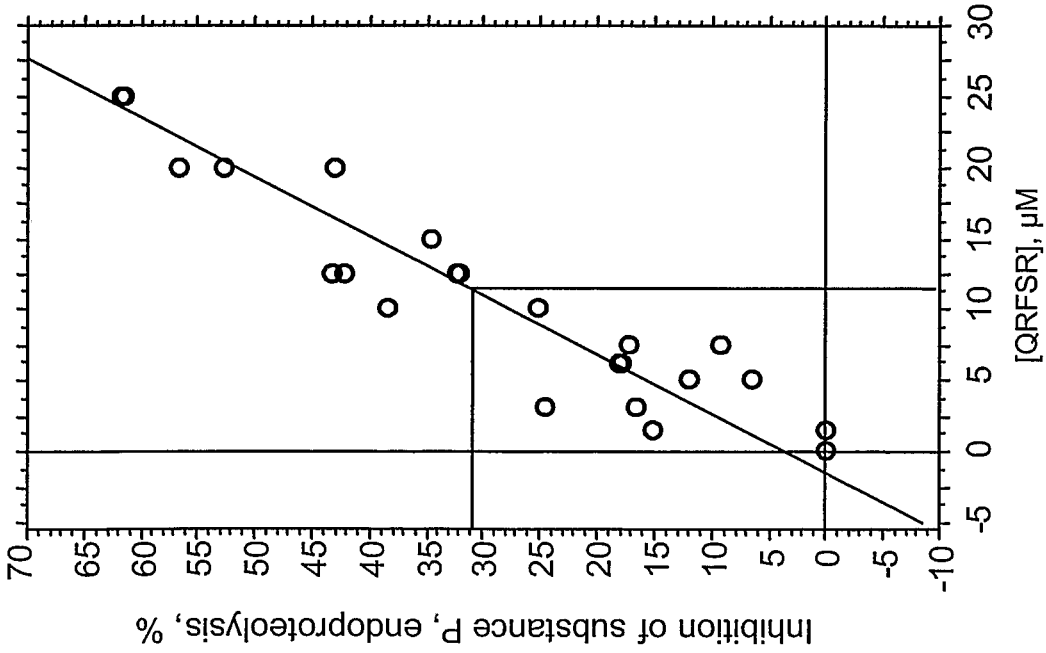
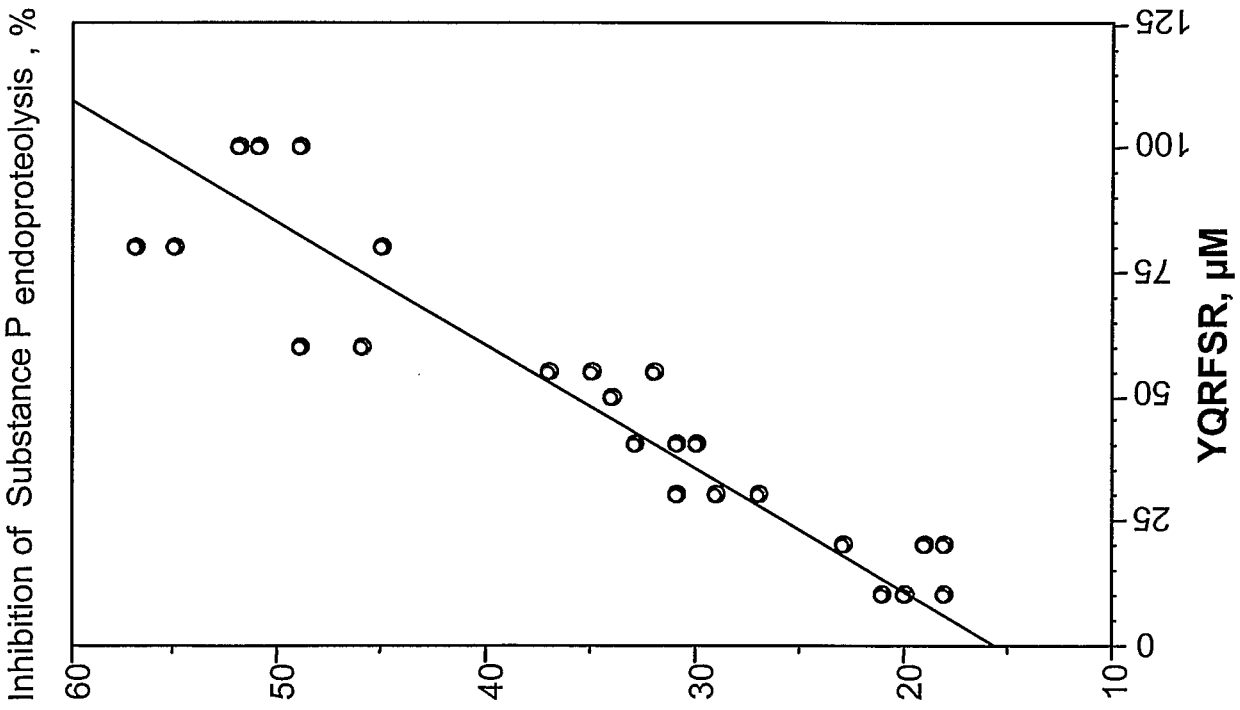
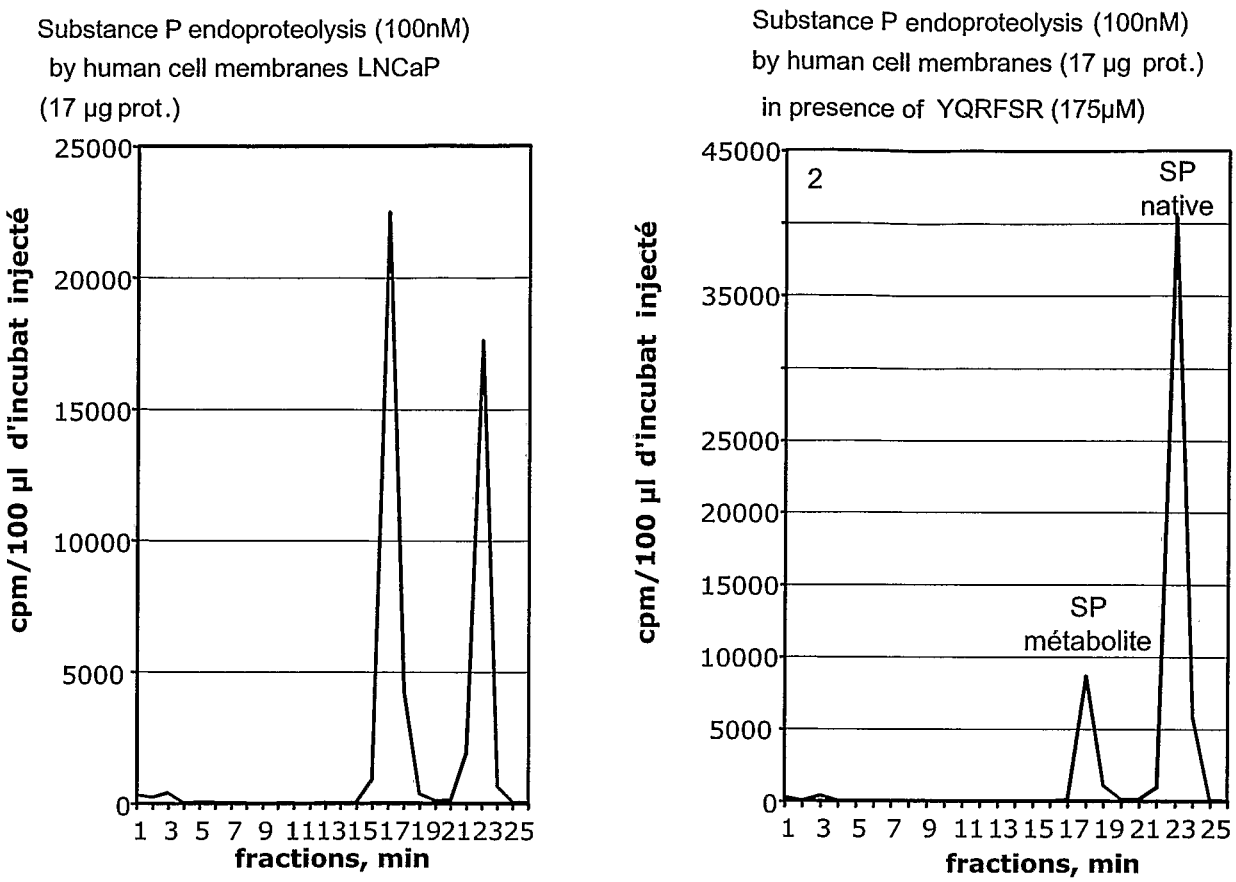

Inhibition of substance P, endoproteolysis, %
[QRFSR], µM
-10
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70

FIG.5

FIG.7


Inhibition of Substance P endoproteolysis , %
60
50
40
30
20
10
125
100
75
50
25
0
YQRFSR, µM


Substance P endoproteolysis (100nM)
by human cell membranes LNCaP
(17 µg prot.)
cpm/100 µl d'incubat injecté
25000
20000
15000
10000
5000
0
1 3 5 7 9 11 13 15 17 19 21 23 25
fractions, min
Substance P endoproteolysis (100nM)
by human cell membranes (17 µg prot.)
in presence of YQRFSR (175µM)
cpm/100 µl d'incubat injecté
45000
35000
25000
20000
15000
10000
5000
0
2
SP
native
SP
métabolite
1 3 5 7 9 11 13 15 17 19 21 23 25
fractions, min

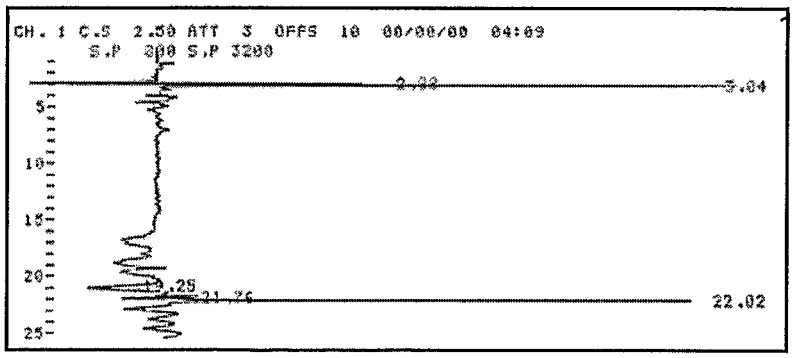

CH. 1 C.S 2.50 ATT 3 OFFS 10 00/00/00 04:09
S.P 200 S.P 3200
2.00
5.04
5
10
15
20
25
19.25
21.26
22.02

FIG.8

FIG.9

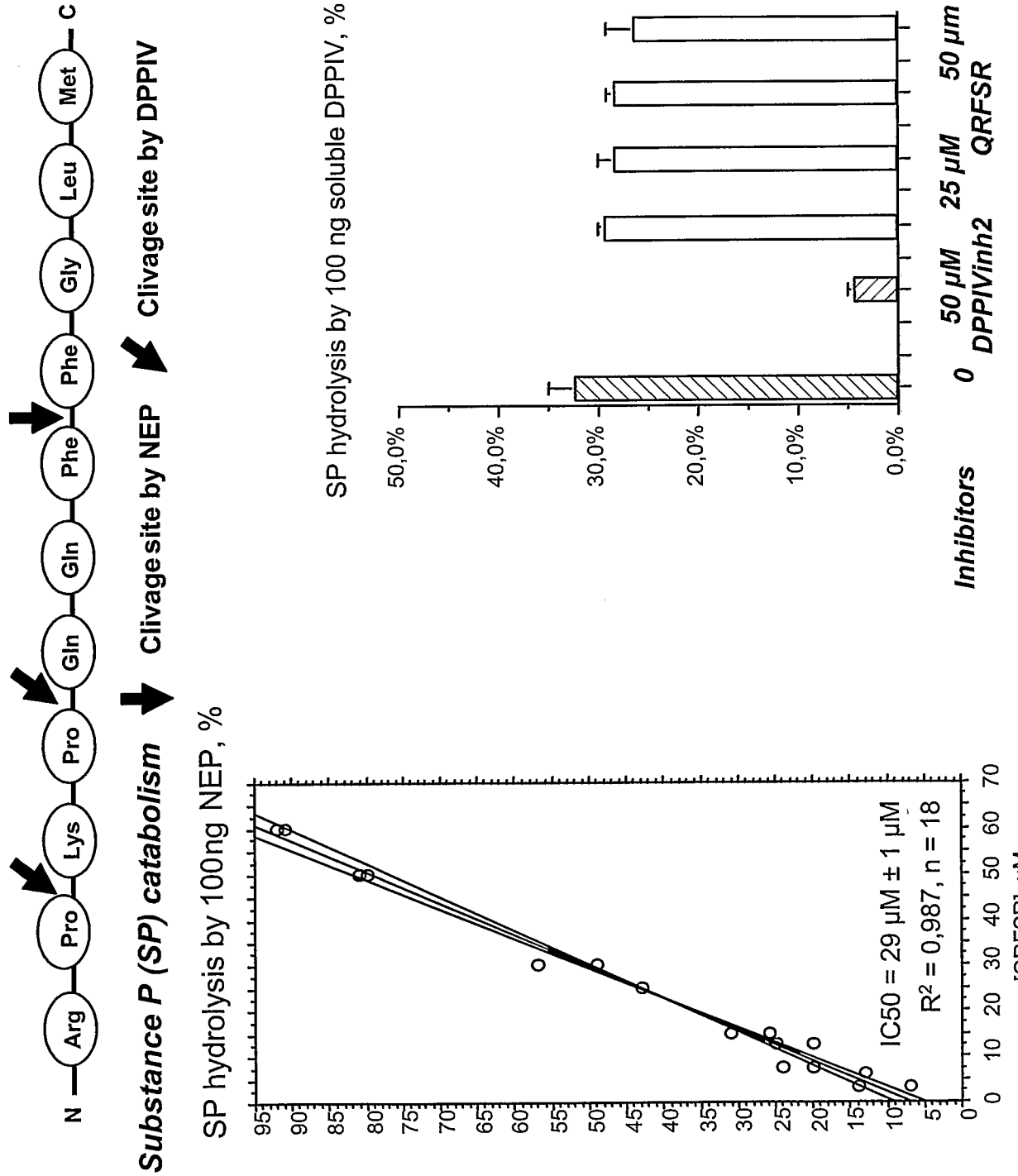

N
Arg
Pro
Lys
Pro
Gln
Gln
Phe
Phe
Gly
Leu
Met
C
Substance P (SP) catabolism
Clivage site by NEP
Clivage site by DPPIV
SP hydrolysis by 100ng NEP, %
IC50 = 29 µM ± 1 µM
R² = 0,987, n = 18
[QRFSR], µM
SP hydrolysis by 100 ng soluble DPPIV, %
50,0%
40,0%
30,0%
20,0%
10,0%
0,0%
Inhibitors
0
50 µM DPPIVinh2
25 µM QRFSR
50 µm

FIG.10

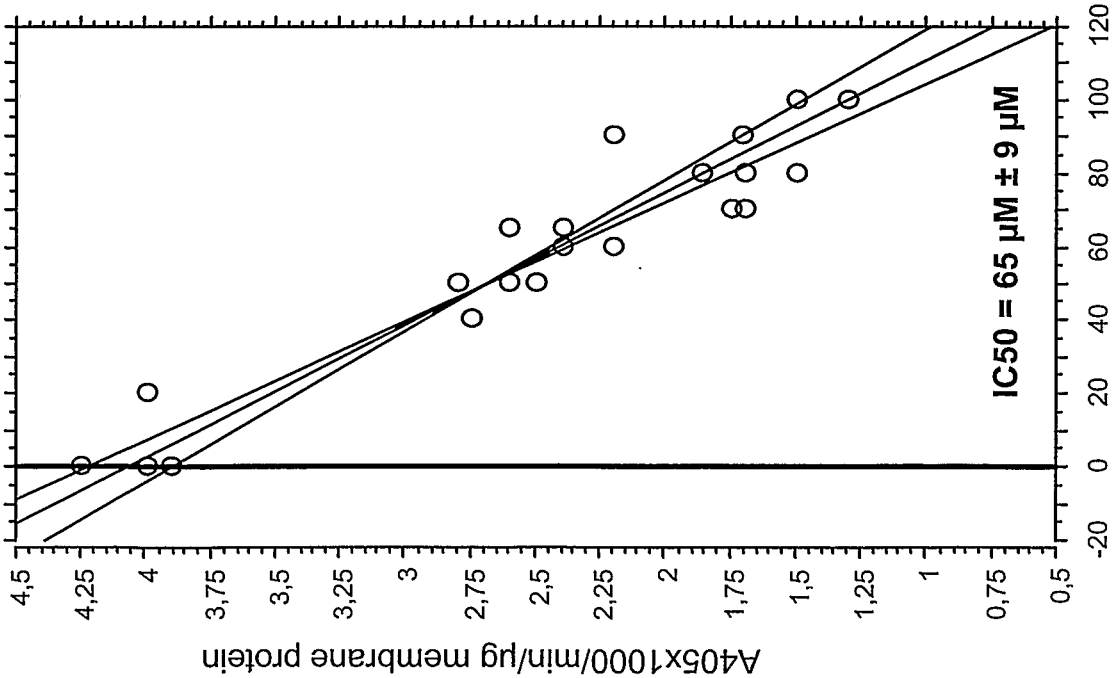
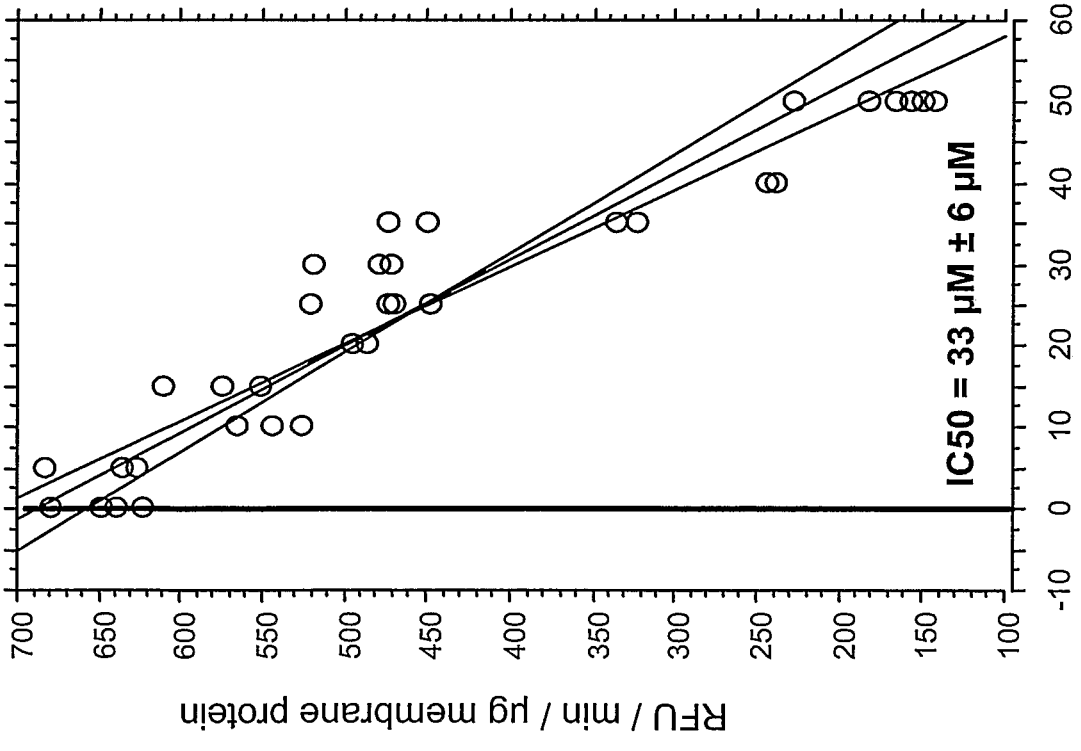

A405x1000/min/µg membrane protein
4,5
4,25
4
3,75
3,5
3,25
3
2,75
2,5
2,25
2
1,75
1,5
1,25
1
0,75
0,5
-20
0
20
40
60
80
100
120
[QRFSR], µM
IC50 = 65 µM ± 9 µM
A405x1000/min/µg membrane protein = 4,066 - ,028 * [QRFSR], µM; R2 = 0,926, n = 22

FIG.11


RFU / min / µg membrane protein
700
650
600
550
500
450
400
350
300
250
200
150
100
-10
0
10
20
30
40
50
60
[QRFSR], µM
IC50 = 33 µM ± 6 µM
RFU / min / µg membrane protein = 686,972 - 9,404 * [QRFSR], µM; R2 = 0,884, n = 29

| Pain behavior: Duration of paw licking | Vehicle (n = 8) | 0.5 mg/kg I.V. (n = 8) | 1 mg/kg I.V. (n = 8) |
|---|---|---|---|
| during the 0-20 min phase of formalin test F(2,21) = 4.42 ; p = 0.025 | 144.13 ± 17.42 | 97.13 ± 13.70* t = 2.12 ; p =0.052 | 84.25 ± 13.53* t = 2.71 ; p= 0.017 |
| 20-40 min. phase F(2,21) = 3.36 ; p = 0.054 | 175.13 ± 10.45 | 123.50 ± 15.04* t = 2.82 ; p= 0.014 | 121.13 ± 22.26* t = 2.20 ; p= 0.045 |
| 40-60 min. phase F(2,21) = 15.37 ; p = 0.0001 | 63.00 ± 12.65 | 12.13 ± 3.44* t = 3.88 ; p= 0.002 | 8.75 ± 2.90* t = 4.18 ; p= 0.001 |

(*) Dunnett' t test vs vehicle

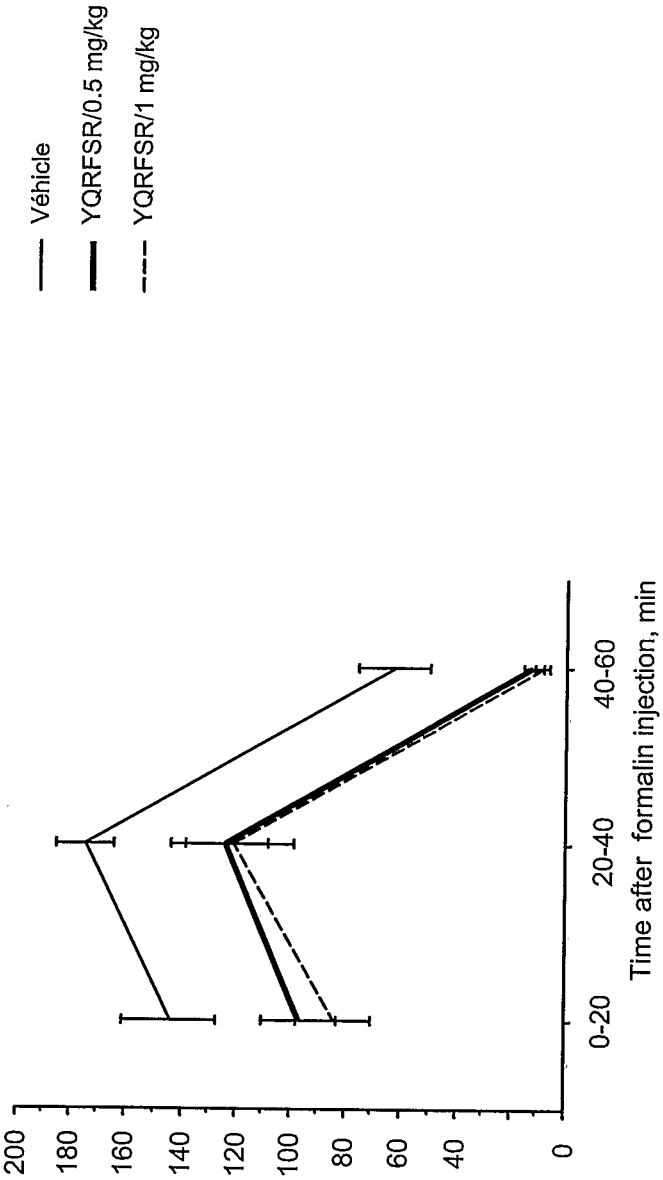



FIG.12

| Pain behavior: Number of spasms | Vehicle (n = 8) | 0.5 mg/kg (n = 8) | 1 mg /kg (n = 8) |
|---|---|---|---|
| during the 0-20 min phase of formalin test F(2,21) = 1.07 ; p = 0.361 | 23.75 ± 5.15 | 33.13 ± 11.25 | 18.13 ± 2.77 |
| 20-40 min. phase F(2,21) = 4.39 ; p = 0.026 | 91.50 ± 18.67 | 79.75 ± 9.59 | 39.13 ± 8.67* t = 2.54 ; p= 0.023 |
| 40-60 min.phase F(2,21) = 8.29 ; p = 0.002 | 126.38 ± 13.69 | 103.75 ± 13.86 | 60.88 ± 4.60* t = 4.54 ; p= 0.005 |

(*) Dunnett' t test vs vehicle

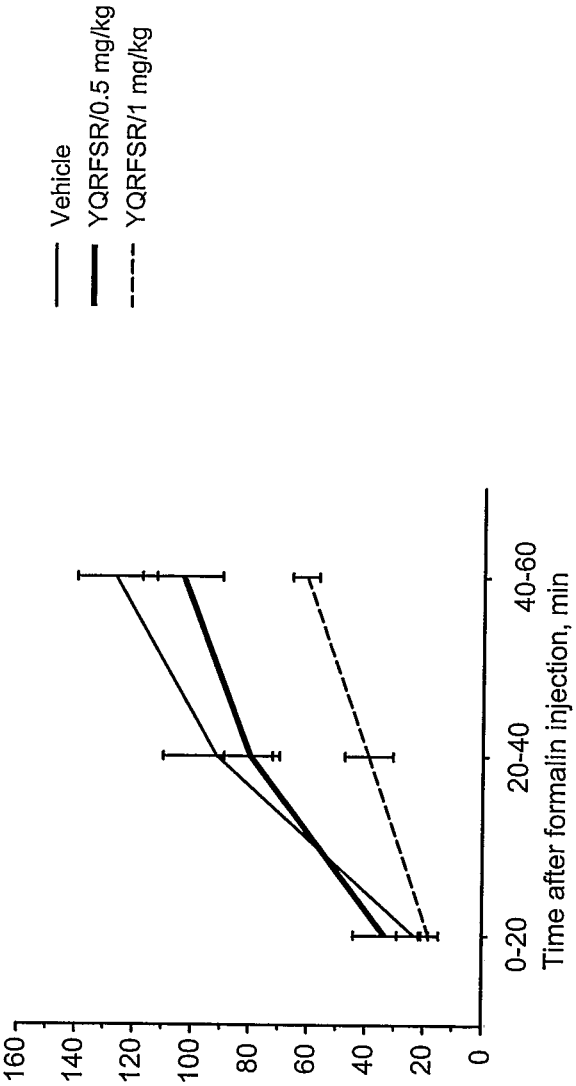



FIG.13

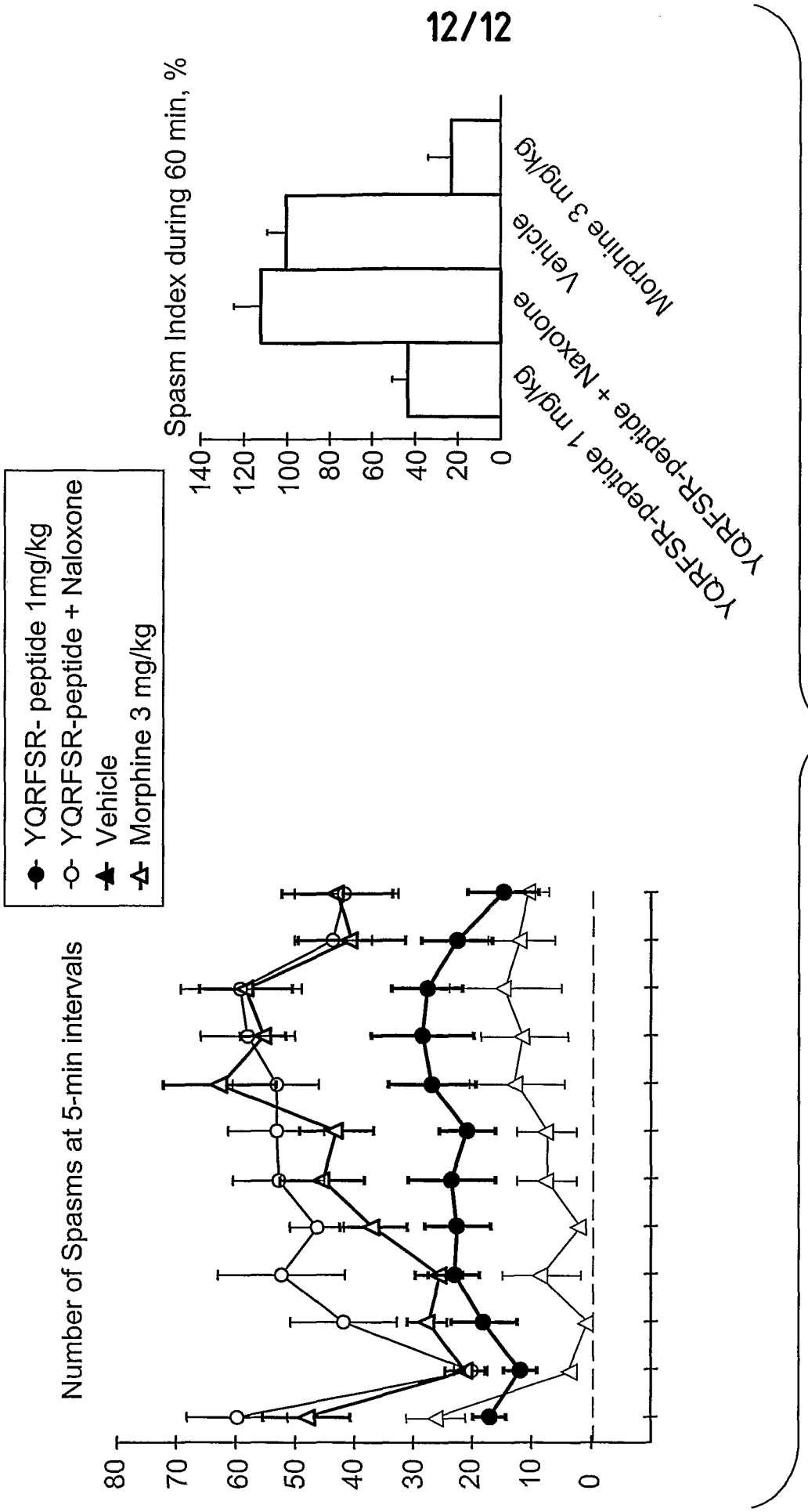

YQRFSR-peptide 1 mg/kg
YQRFSR-peptide + Naxolone
Vehicle
Morphine 3 mg/kg
Spasm Index during 60 min, %
0
20
40
60
80
100
120
140
YQRFSR- peptide 1mg/kg
YQRFSR-peptide + Naloxone
Vehicle
Morphine 3 mg/kg
Number of Spasms at 5-min intervals
0
10
20
30
40
50
60
70
80

FIG.14