British Journal of Pharmacology (1997) 122, 715–725

Localization of Fos-like immunoreactivity induced by the NK_3 tachykinin receptor agonist, senktide, in the guinea-pig brain

Jane Yip & [1]Loris A. Chahl

Neuropharmacology Laboratory, Faculty of Medicine and Health Sciences, The University of Newcastle, N.S.W. Australia 2308

1 The effects of intracerebroventricular (i.c.v.) administration of the NK_3 tachykinin receptor agonist, senktide (10 nmol each side), in guinea-pigs pretreated with the selective NK_3 tachykinin receptor antagonist, SR142801 (3 mg kg^{-1} subcutaneous, s.c., 30 min before senktide), or its less active enantiomer, SR142806 (3 mg kg^{-1} s.c. 30 min before senktide), on behaviour and on the distribution of Fos-like immunoreactivity (Fos-LI) in central neurones were investigated. Guinea-pigs were chosen for the study since they possess NK_3 tachykinin receptors with pharmacological characteristics similar to those in man.

2 Wet-dog shakes, but not locomotor activity, elicited by senktide i.c.v. were significantly reduced by SR142801 but not by SR142806, confirming the involvement of NK_3 tachykinin receptors in wet-dog shake behaviour.

3 Senktide induced increased numbers of Fos-LI neurones in the following brain areas: frontal, parietal and piriform cortex, the lateral septum, the CA1, CA2, subiculum and dentate gyrus of the hippocampus, most areas in the amygdala, thalamus and hypothalamus, medial geniculate nucleus and the ventral cochlear nucleus. Pretreatment with SR142801, but not with SR142806, before administration of senktide inhibited Fos-LI expression in the cingulate cortex, dentate gyrus of the hippocampus, some regions of the thalamus, hypothalamus and amygdala and the ventral cochlear nucleus.

4 The present results are the first demonstration that senktide induces Fos-LI in widespread areas of the guinea-pig brain. It is proposed that NK_3 tachykinin receptors may play a more extensive role in the control of diverse brain functions, including cortical processing, learning and memory, neuroendocrine and behavioural regulation, than is currently recognized.

Keywords: Tachykinin; NK_3 tachykinin receptor antagonist; locomotor activity; wet-dog shakes; Fos-immunoreactivity; intracerebroventricular; guinea-pig brain

Introduction

The tachykinins are biologically active peptides, of both mammalian and nonmammalian origin, which all possess the same carboxyl terminal sequence, Phe-X-Gly-Leu-Met-NH_2. The mammalian tachykinins substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) are found in the peripheral and central nervous systems (CNS) where they have been implicated as neurotransmitters, neuromodulators and neurotrophins (Otsuka & Yoshioka, 1993). Three tachykinin receptors, termed NK_1, NK_2 and NK_3, have been functionally identified and cloned (Nakanishi, 1991; Regoli *et al*., 1994). A major advance in the last five years has been the development of nonpeptide tachykinin receptor antagonists selective for each receptor type. The availability of nonpeptide tachykinin antagonists has led to the discovery of species differences in tachykinin receptors (Fardin & Garret, 1991; Maggi 1995; Suman-Chauhan *et al*., 1994). The types of NK_1, NK_2 and NK_3 tachykinin receptors in guinea-pigs appear to be similar to those in man, but differ from those in rats (Maggi, 1995; Regoli *et al*., 1994; Emonds-Alt *et al*., 1995).

It is now known that NK_1, NK_2 and NK_3 tachykinin receptors are present in the mammalian brain (Dam & Quirion, 1994). Studies on the central distribution of NK_3 tachykinin receptors have been largely performed in rats (Marksteiner *et al*., 1992; Dam & Quirion, 1994; Ding *et al*., 1996), the only study in guinea-pigs being that of Dam and Quirion (1994). NK_3 tachykinin receptors have been found in guinea-pig cortex, hippocampus and striatum (Dam & Quirion, 1994).

Earlier studies in rats performed by Elliott and Iversen (1986) described behavioural responses, comprising increased locomotor activity, wet-dog shakes and grooming, induced by centrally administered substance P and various analogues. Subsequent studies with the selective NK_3 tachykinin receptor agonist, senktide, administered intracerebroventricularly (i.c.v.), showed wet-dog shakes in rats and head-twitching in mice (Stoessl *et al*., 1988) and increased locomotor activity (Johnston & Chahl, 1993) and wet-dog shakes (Piot *et al*., 1995) in guinea-pigs. Until recently, the lack of potent and selective NK_3 tachykinin receptor antagonists has hindered studies on the role of these receptors in physiological functions. Therefore the development of the recently described NK_3 tachykinin receptor antagonist, SR142801 (Emonds-Alt *et al*., 1995), represents an advance in the field of tachykinin research. SR142801 is highly species-dependent having a higher affinity for NK_3 tachykinin receptors present in man, gerbils and guinea-pigs than in rats (Emonds-Alt *et al*., 1995).

Currently, the brain regions activated by senktide to produce behavioural changes remain unexplored. A useful method for obtaining a functional map of the neurones activated by drugs is to use immunohistochemical localization of Fos, the protein product of the immediate-early gene transcription factor, *c-fos*, which is induced in nuclei of activated neurones (Morgan & Curran, 1991). Although Fos protein is found in the CNS following application of a wide range of stimuli to animals, previous studies with opioid agonists have demonstrated that it is not a nonspecific marker and that different patterns of Fos activation are seen following treatment of animals with different agonists (Bot & Chahl, 1996).

In this study, the distribution of neurones expressing Fos-like immunoreactivity (Fos-LI) in the guinea-pig brain following i.c.v. administration of senktide is described. Guinea-

[1] Author for correspondence.

pigs rather than rats were chosen for the study since guinea-pigs possess NK_3 tachykinin receptors which pharmacologically resemble human NK_3 tachykinin receptors. Furthermore, the effect of the NK_3 tachykinin receptor antagonist, SR142801, on the distribution of Fos-LI neurones was investigated, to determine the functional involvement of NK_3 tachykinin receptors in the actions of senktide in the guinea-pig brain. Since tachykinins are found in high concentrations in areas of the brain rich in dopamine-containing neurones, double-labelling studies were also carried out to determine the extent of Fos-LI induction in neurones containing tyrosine hydroxylase, the rate limiting enzyme in the synthesis of dopamine and other catecholamines.

Methods

Animal preparation

Adult guinea-pigs of either sex weighing 450 – 550 g were used. Animals were housed in a room maintained at constant temperature (23°C) and on a 12 h light/dark cycle. Food (standard guinea-pig pellets) and water were available *ad libitum* and ascorbic acid was added to the drinking water daily.

Implantation of intracerebroventricular cannulae was carried out as previously described (Johnston & Chahl, 1991; Bot *et al*., 1992). Briefly, guinea-pigs were anaesthetized with ketamine hydrochloride (Ketalar, 40 mg kg^{-1}, s.c.) and xylazine (Rompun, 4 mg kg^{-1}, s.c.). By means of aseptic techniques stainless steel guide cannulae (i.d. 0.3 mm, o.d. 0.5 mm, 6 mm in length) were inserted bilaterally 2 mm caudal and 2.5 mm lateral to bregma and to a depth of 1 mm, and glued to the skull. The animals were caged individually and given at least 4 days to recover before use in experiments. Care was taken to minimize stress to the guinea-pigs. Animals were handled daily by the same experimenter and habituated to the test cages for 30 min daily for 5 days before the experiment to minimize stress. These test cages contained shavings on the floor similar to home cages. The activity of control animals was low, which allowed excellent detection of increase in activity. The presence of behaviours was recorded by a trained observer throughout the test period following drug administration. The scores obtained by the trained observer were counter-checked on several occasions by a disinterested observer who was not acquainted with the expected outcomes of the experimental treatments.

On the day of the experiment, guinea-pigs were observed for the 1 h habituation period and then randomly allocated to control and treatment groups. There were at least four animals per group. In treatment groups, animals were given injections of senktide, 10 nmol in 10 μl of 20 mM acetic acid, into each lateral ventricle (total dose 20 nmol). Lower doses were not used since in a pilot experiment a lower dose of senktide (0.625 nmol into each ventricle) did not increase locomotor activity and produced only infrequent wet-dog shakes. The NK_3 tachykinin receptor antagonist, SR 142801, or its inactive enantiomer, SR142806, at a dose of 3 mg kg^{-1}, was given s.c. 30 min before the i.c.v. injections of senktide (Emonds-Alt *et al*., 1995). Other animals were given 10 μl of 20 mM acetic acid into each lateral ventricle and a s.c. injection of 0.2 ml of 0.01% Tween 80 to control for the effects of the vehicles for senktide and SR142801, respectively. Immediately after drug administration, animals were placed into photocell cages and observed for 90 min.

Locomotor and behavioural activity of guinea-pigs were measured in a modified animal cage equipped with a single infra-red photocell and detector on the longitudinal axis. A digital counter recorded every crossing of the infra-red beam at least 1.5 s apart and a single pulse record was made simultaneously on a chart recorder. Activity scores were obtained from the total number of counts over successive 10 min intervals throughout the experiment. At the end of the 90 min observation period, guinea-pigs were injected with sodium pentobarbitone (80 mg kg^{-1}) and perfuse-fixed with 4% paraformaldehyde in phosphate buffer. The brains were removed, post-fixed for 2 h and placed in 30% sucrose solution in 0.5% paraformaldehyde for cryoprotection.

Staining procedures

Brain tissue was mounted on a microtome, covered in tissue freezing medium (Triangle Biomedical Sciences, Durham, North Carolina, U.S.A.) and frozen at −18°C in a cryostat. Coronal sections of 30 μm thickness were cut and one in five sections was taken and floated in a multi-well tray containing phosphate-buffered saline (50 mM PBS). After the entire brain had been cut, the tissues were placed on a rocking table for 1 h before PBS was replaced. Endogenous peroxidase activity was inhibited by washing sections in PBS containing 0.3% hydrogen peroxide for 20 min. Sections were incubated in 50 mM PBS with 3% nonfat milk (Sigma Immunochemicals, Sigma Chemical Company, St. Louis, Missouri, U.S.A.) and 1% BSA to block nonspecific protein interactions for 1 h at 4°C. Following a 15 min wash in Triton diluent (PBS containing 0.075% Triton X-100, 0.1% BSA, 0.01% sodium azide), the sections were incubated with sheep polyclonal antibody to Fos oncoprotein (Cambridge Research Biochemicals, Northwich, Cheshire, U.K.) either alone for single-staining, or for double-staining, together with monoclonal antibody to tyrosine hydroxylase (Boehringer Mannheim Biochemica, Mannheim, Germany), both at a dilution of 1 in 1000 in Triton diluent, at 4°C for at least 48 h. Fos is a 55 kDa nuclear associated protein. The Fos antiserum was raised against a synthetic peptide selected from a conserved region of human c-Fos common to several members of the Fos family. Therefore, the antiserum recognizes Fos (62 kDa), Fos Related Antigens (48/49 and 70 kDa) as well as other transcription factors that contain leucine zipper structures such as members of the ATF (activating transcription factor)/CRE (cyclicAMP responsive element) protein families (Hai & Curran, 1991). Sections were then washed 3 times in Triton diluent and incubated overnight at 4°C in biotinylated donkey anti-sheep antibody at a dilution of 1 in 500 (secondary antibody for Fos antiserum) (Jackson ImmunoResearch Laboratories, Inc. West Grove, Pennsylvania, U.S.A.). They were then washed 5 times in PBS and incubated overnight at 4°C in avidin-biotin horseradish peroxidase complex (ABC). The ABC solution consisted of 100 μl A and B reagents in 100 ml PBS previously reacted together for 30 min before use (ABC *Elite* kit from Vector Laboratories, Burlingame, California, U.S.A.). Following 3 washes in 50 mM Tris buffer the trays were placed on ice and the antigen-antibody complexes were visualized by the addition of 3,3′-diaminobenzidine (DAB) containing nickel and cobalt (0.033% DAB in 50 mM Tris buffer containing 0.01% nickel sulphate, 0.007% cobalt chloride, and 0.03% hydrogen peroxide) (Hsu & Soban, 1982). The solution was filtered through Watman no. 1 filter paper before use to eliminate particulate matter. Once optimal stain intensity was achieved, the reaction was terminated with the addition of excess cold distilled water and sections washed again twice in cold distilled water. Sections to be double-stained for Fos and tyrosine-hydroxylase, were then incubated in antimouse antibody (1 in 500, Jackson ImmunoResearch Laboratories, Inc. West Grove, Pennsylvania, U.S.A.) overnight at 4°C, washed 5 times in PBS and incubated in ABC solution as described previously. Following 3 washes in 50 mM Tris buffer the trays were placed on ice and stained with DAB solution without nickel and cobalt (0.066% DAB and 0.03% hydrogen peroxide in 50 mM Tris buffer). After staining, the sections were mounted on chrome-alum coated slides and air-dried overnight before being dehydrated in graded ethanol solutions, cleared with Histoclear (National Diagnostics Atlanta, U.S.A.) and coverslipped with Ultramount (Histo-Labs, Fronine Pty Ltd, Riverstone, NSW, Australia). Control sections were incubated without one or both primary antibodies and/or secondary antibodies.

Quantitative Fos and tyrosine hydroxylase immunohistochemistry

To compare either Fos-LI or tyrosine hydroxylase staining in the guinea-pig brain following each of the treatments, one section with optimal staining from each selected brain region was chosen for each animal and the number of Fos-LI positive neurones was counted under bright-field microscopy. Mean values for the number of Fos-LI neurones for each treatment group were calculated. The nomenclature for brain regions and regional boundaries used was extrapolated from those described for rat brain in the atlas of Paxinos and Watson (1986), since no detailed atlas of guinea-pig brain is available. For areas with clear landmarks such as the cingulate cortex or caudate putamen, it was easy to gauge comparable boundaries. However, for some areas such as regions of the amygdala and the thalamus where boundaries were less obvious, the number of Fos-LI stained cells was counted within a set number of fields of the microscope. The counting procedure was verified by an independent observer to eliminate operator bias.

Drugs

The following drugs were used: ketamine hydrochloride 100 mg ml^{-1} (Ketalar, Parke-Davis, Australia), senktide (Auspep Pty Ltd, Australia), SR142801 (**S**)-(N)-(1-(3-(1-ben-

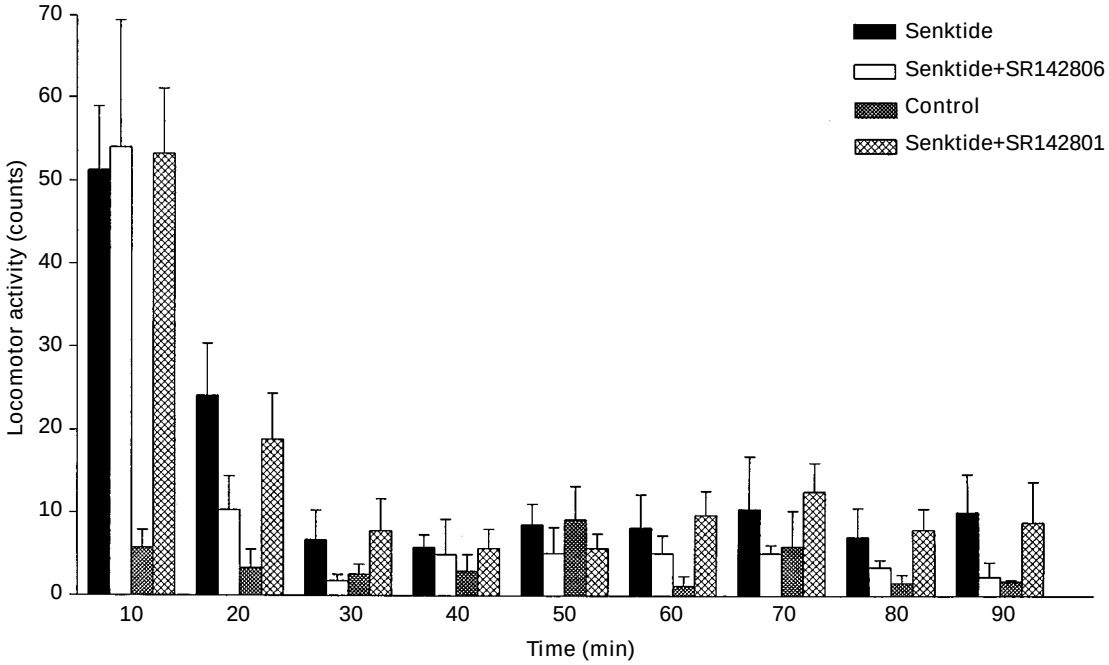

Figure 1 Effects of senktide on locomotor activity in guinea-pigs. Locomotor scores were obtained by adding the total number of counts over successive 10 min periods. Histograms represent means obtained for the number of animals given below in parentheses and the vertical lines are s.e.means. Solid columns represent results for animals treated with senktide 10 nmol, i.c.v., each side ($n=7$), open columns from guinea-pigs treated with the inactive enantiomer of the NK_3 tachykinin receptor antagonist, SR142806 3 mg kg^{-1}, s.c., 30 min before senktide ($n=5$), and cross-hatched columns from guinea-pigs treated with the active enantiomer of the NK_3 tachykinin receptor antagonist, SR142801 3 $mgkg^{-1}$, s.c., 30 min before senktide ($n=8$). Stippled columns represent the control group given s.c. injection of vehicle, 0.2 ml of 0.01% Tween 80 30 min before an i.c.v. injection of 10 μl acetic acid each side ($n=4$).

Table 1 Effects of SR142801 and SR142806 on behavioural responses of guinea-pigs to senktide

	Control	*Senktide + SR142806*	*Senktide*	*Senktide + SR142801*
WDS				
10 min	0.0±0.0	26.2±6.0	24.6±4.4	*†13.1±2.3
20 min	0.0±0.0	7.8±7.5	3.6±2.4	2.1±0.9
C&T				
10 min	2.8±1.7	30.6±4.9	26.3±6.8	31.3±6.6
20 min	1.8±1.0	13.4±3.8	8.9±6.8	14.7±5.2
FW&G				
10 min	0.0±0.0	0.2±0.2	0.1±0.1	0.3±0.2
20 min	0.0±0.0	1.0±0.8	1.4±0.4	0.1±0.1

Numbers show mean values±s.e.means for behavioural responses of guinea-pigs over the first 20 min following i.c.v. injections of senktide (10 nmol, each side, in 10 µl of 20 mM acetic acid) in untreated animals ($n=7$), and animals pretreated s.c. 30 min before senktide with SR142801 (3 mg kg^{-1} in 0.01% Tween 80) ($n=8$), or SR142806 (3mg kg^{-1}) ($n=5$). Control guinea-pigs were given s.c. injections of 0.2 ml of 0.01% Tween 80 30 min before the i.c.v. injections of 10 µl each side of 20 mM acetic acid ($n=4$). Wet-dog shake behaviour induced by senktide was significantly reduced by SR142801 (*$P=0.033$ for comparison between untreated and SR142801 treated guinea-pigs); (†$P=0.038$ for comparison between SR142801 and SR142806 treated guinea-pigs). Abbreviations: WDS, wet-dog shakes; C&T, circling around the perimeter of the cage and turning on the spot; FW&G, face-washing and grooming.

Table 2 Semiquantitative representation of the numbers of neurones showing Fos-LI 90 min after i.c.v. administration of senktide

Brain area	*Control*	*Senktide*
Telencephalon		
Claustrum	+ +	+ + + +
Tenia tecta	+	+ +
Neocortex		
Frontal	+ + +	+ + + +
Parietal	+ + +	+ + + +
Temporal	+ + +	+ + + +
Occipital	+ + +	+ + + +
Allocortex		
Piriform	+ + +	+ + + +
Orbital	+ + +	+ + + +
Infralimbic	+ + +	+ + + +
Cingulate	+ + +	+ + + +
Insular	+ + +	+ + + +
Septal area		
Accumbens nucleus (n)	+	+ +
Lateral septal n.	+ +	+ + + +
Medial septal n.	+	+ +
Striatum		
Caudate putamen	+ +	+ + +
Globus pallidus	+	+ +
Bed nucleus stria terminalis	+	+ +
Septohypothalamic n.	+ +	+ + +
Septohippocampal n.	+ +	+ + +
Hippocampal formation		
CA1	+	+ +
CA2	+ +	+ + +
CA3-CA4	+	+
Dentate gyrus	+ +	+ + + +
Subiculum	+ +	+ + +
Amygdaloid nuclei		
Medial	+ +	+ + + +
Posteromedial	+ +	+ + +
Posterolateral	+	+ + +
Posterocortical	+ +	+ + +
Basolateral	+ +	+ + +
Basomedial	+ +	+ + +
Diencephalon		
Thalamus		
Intermedial dorsal n.	+ +	+ + + +
Posteromedian n	+ +	+ + + +
Central medial n.	+ +	+ + + +
Medial dorsal n.	+ +	+ + + +
Parafascicular n.	+	+ +
Paraventricular n.	+	+ +
Ventral lateral n.	+	+ +
Lateral dorsal n.	+	+ +
Zona Incerta	+	+ +
Mamillothalamic tract	+	+ +
Medial lemniscus	+	+ +
Medial geniculate n.	+	+ +
Lateral geniculate n.	+	+
Dorsolateral geniculate n.	+	+ +
Medial habenula n.	+	+ + +
Lateral habenula n.	+	+ + +
Hypothalamus		
Medial preoptic n.	+	+ +
Lateral preoptic n.	+	+ +
Supraoptic n., retrochiasmatic	+	+
Periventricular n.	+	+ +
Paraventricular n.	+	+
Anterior hypothalamic area	+	+
Ventromedial	+	+ + +
Dorsomedial	+	+ + +
Arcuate n.	+	+ +
Lateral hypothalamic area	+	+ +
Magnocellular n., lateral hypothal.	+	+ +
Dorsal hypothalamic area	+	+ +
Gemini hypothalamic n.	+	+ +
Median eminence	+ +	+ + +

Table 2 continued opposite

zoyl-3-(3,4-dichlorophenyl) piperidin-3-yl) propyl)-4-phenyl-piperidin-4-yl)-N-methylacetamide) and its (**R**)-enantiomer SR142806 (kindly donated by Dr Emonds-Alt of Sanofi Recherche, France), xylazine 20 mg ml^{-1} (Rompun, Bayer Australia Ltd, Botany, NSW, Australia) and xylocaine hydrochloride 5 mg ml^{-1} (Astra Pharmaceuticals Pty Ltd, Australia). Senktide was dissolved in 20 mM acetic acid and stored frozen ($-30°C$) in 20 μl aliquots. SR142801 and SR142806 were dissolved in 0.01% Tween 80 and stored as aliquots at $-30°C$. On the day of experiment, the drugs were thawed from the aliquots and used immediately without dilution.

Statistics

Student's *t* tests were used to compare the effects of drug treatments on the number of stained neurones in particular brain regions with controls and with each other.

Results

Effect of senktide on behaviour

Increased locomotor activity and other behavioural effects such as wet-dog shakes were observed in guinea-pigs given an i.c.v. injection of senktide, 10 nmol each side. These behaviours commenced rapidly after senktide administration and subsided after 20 min. The locomotor activity, shown graphically in Figure 1, consisted mainly of circling around the periphery of the cage and turning around on the spot. Data for circling and turning are presented quantitatively in Table 1. Other behaviours which occurred included wet-dog shakes and face-washing and grooming (Table 1) and more rarely chewing, eating, head-lifting and cage-biting (data not shown). Control animals showed little locomotor activity throughout the observation period and did not show wet-dog shakes, face-washing and grooming or other behaviours. Guinea-pigs that were treated 30 min before senktide administration with a s.c. injection of either SR142801 or SR142806 demonstrated increased locomotor activity similar to that observed for senktide (Figure 1). However, the senktide-induced wet-dog shakes in the first 10 min were significantly reduced by the NK_3 tachykinin receptor antagonist, SR142801 (Table 1, $P=0.033$,

Table 2 *continued*

Brain area	*Control*	*Senktide*
Mesencephalon		
Medial lemniscus	+	+ + +
Interpeduncular	+ +	+ + +
Ventral Tegmental Area	+	+
Substantia nigra compacta	+	+
Substantia nigra reticulata	+	+
Periaqueductal gray	+	+
Superior Colliculus	+ + +	+ + +
Red nucleus	+	+
Metencephalon		
Cerebellar lobules	+ +	+ + +
Pontine reticular n.	+	+
Lateral superior olive	+	+
Lateral parabrachial n.	+	+
Locus coeruleus	+	+
Dorsal Raphe	+	+ +

The relative abundance of Fos-LI in different regions of the guinea-pig brain determined visually (by use of bright field microscopy) from comparable sections with the strongest staining that is representative of the average results obtained for all animals in the group. + + + +, very high; + + +, high; + +, moderate; +, low. Levels reflect the relative densities of neurones.

Table 3 Effects of SR142801 and SR142806 on the number of neurones expressing Fos-LI in guinea-pig brain regions after administration of senktide

Area	*Control (vehicle)*	*Senktide*	*Senktide + SR142801*	*Senktide + SR142806*
Cortex				
Frontal 1 and 2	418.0 ± 32.7	815.0 ± 52.1	687.2 ± 44.8	634.2 ± 78.7
Cingulate 1	139.5 ± 25.6	221.8 ± 14.1	59.2** ± 3.1	207.4 ± 32.6
Cingulate 2	122.7 ± 29.5	183.6 ± 23.4	30.4*** ± 11.3	223.8 ± 27.6
Lateral septal nucleus (n.)	98.2 ± 17.0	381.6 ± 13.7 ($n=5$)	82.4* ± 21.3 ($n=5$)	206.8 ± 35.6
Islands of Calleja	68.5 ± 11.1	268.2 ± 32.0 ($n=5$)	85.2†† ± 12.0 ($n=4$)	160.4 ± 32.6
Striatum				
Globus pallidus	28.2 ± 5.8	60.1 ± 6.7 ($n=6$)	41.4 ± 8.4 ($n=5$)	47.8 ± 8.8
Caudate putamen	489.2 ± 142.6	1302.3 ± 257.0 ($n=6$)	832.8 ± 88.9	1092.2 ± 74.0
Hippocampus				
Dentate gyrus	207.8 ± 46.8	405.7 ± 34.6	232.4** ± 43.1	508.2 ± 63.1
Amygdala				
Medial amygdaloid n.	143.5 ± 21.7	489.8 ± 82.0 ($n=5$)	146.5††† ± 18.4 ($n=4$)	188.1 ± 31.0
Posteromedial cortical n.	156.2 ± 28.2	314.3 ± 35.5 ($n=6$)	127.6* ± 14.9 ($n=6$)	211.8 ± 22.8
Amygdalahippocampal area	98.25 ± 17.6	181.1 ± 22.2 ($n=6$)	102.1† ± 26.7 ($n=6$)	108.6 ± 12.1
Basomedial amygdaloid n.	141.7 ± 18.5	334.3 ± 51.1 ($n=6$)	77.3* ± 10.7 ($n=6$)	166.5 ± 34.8
Basolateral amygdaloid n.	144.0 ± 20.7	385.8 ± 67.8 ($n=6$)	64.6* ± 10.2 ($n=6$)	164.6 ± 34.8
Thalamus				
Intermediodorsal n.	78.2 ± 8.5	161.2 ± 10.9 ($n=6$)	84.6††† ± 10.0 ($n=6$)	135.6 ± 21.9
Paraventricular n.	41.7 ± 9.1	110.0 ± 6.8 ($n=6$)	80.0† ± 8.6 ($n=6$)	88.6 ± 21.9
Posteromedian & central medial n.	150.4 ± 10.1	383.0 ± 23.2 ($n=5$)	182.0† ± 23.7 ($n=6$)	242.8 ± 29.1
Zona Incerta	96.5 ± 8.6	106.2 ± 9.5 ($n=6$)	77.8 ± 10.6 ($n=5$)	110.6 ± 12.6
Habenula (medial and lateral)	55.4 ± 8.9	211.0 ± 29.1 ($n=6$)	72.0* ± 32.1 ($n=5$)	175.0 ± 25.0
Hypothalamus				
Lateral n.	48.9 ± 8.6	83.2 ± 9.7 ($n=6$)	57.8 ± 8.2 ($n=6$)	75.2 ± 7.4
Dorsomedial n.	60.1 ± 6.6	159.4 ± 15.0 ($n=5$)	77.5* ± 7.1 ($n=6$)	108.6 ± 12.4
Mesenephalon				
Substantia nigra pars reticulata	50.1 ± 11.3	59.0 ± 9.2	54.5 ± 9.2	66.8 ± 6.9
Substantia nigra pars compacta	28.7 ± 5.6	28.1 ± 8.0	26.5 ± 4.8	33.4 ± 8.4
Ventral tegmental area	14.6 ± 5.5	23.3 ± 4.2	22.1 ± 4.6	15.8 ± 3.3
Interpeduncular n.	177.2 ± 8.3	335.5 ± 36.0	265.2 ± 19.4	295.8 ± 14.6
Superior colliculus	408.2 ± 44.5	482.8 ± 56.4	433.0 ± 26.1	519.0 ± 67.8
Metencephalon				
Locus coeruleus	55.4 ± 8.9	83.8 ± 9.5 ($n=5$)	81.8 ± 6.8 ($n=5$)	78.2 ± 4.5
Ventral cochlear n.	570.0 ± 25.7	650.0 ± 40.2 ($n=6$)	443.5* ± 60.1 ($n=6$)	660.7 ± 37.5

Values are mean numbers of neurones ± s.e.means expressing Fos-LI in the guinea-pig brain 90 min following i.c.v. injections of senktide (10 nmol, each side) in untreated guinea-pigs ($n=7$), and in guinea-pigs pretreated 30 min before senktide with SR142801 (3 mg kg^{-1} s.c.) ($n=7$)) or SR142806 (3 mg kg^{-1} s.c.) ($n=5$). Control animals were given 0.2 ml of 0.01% Tween 80 30 min before the i.c.v. injections of 10 µl each side of 20 mM acetic acid ($n=4$). Where results were not available for every animal in a group the number of animals from which results were obtained is shown in parentheses. Analysis was made from comparable sections with strongest staining visualized under bright-field microscopy. Asterisks show levels of significance for comparisons between senktide in animals pretreated with SR142801 and with SR142806. Daggers show levels of significance for comparisons between senktide alone and with SR142801. *or †, $0.05 > P > 0.01$; **or ††, $0.01 > P > 0.001$; ***or †††, $P < 0.001$.

Student's t test), but not by its less active enantiomer, SR142806. SR142801 did not significantly affect other behaviours such as circling and turning or face-washing and grooming (Table 1).

Effect of senktide on distribution of Fos-LI

Table 2 shows a semi-quantitative assessment of Fos-LI induced in various telencephalic, diencephalic, mesencephalic and metencephalic regions of the guinea-pig brain following control (acetic acid, i.c.v. and Tween 80, s.c.) and senktide (10 nmol, i.c.v. each side) treatments. Quantitative results for the effect of vehicles, and for senktide in untreated animals and those treated with the NK_3 tachykinin receptor antagonist, SR142801, and its less active enantiomer, SR142806, both given 30 min before senktide, on the number of Fos-LI neurones in guinea-pig brain regions are shown in Table 3. For most brain regions, results with the less active enantio-

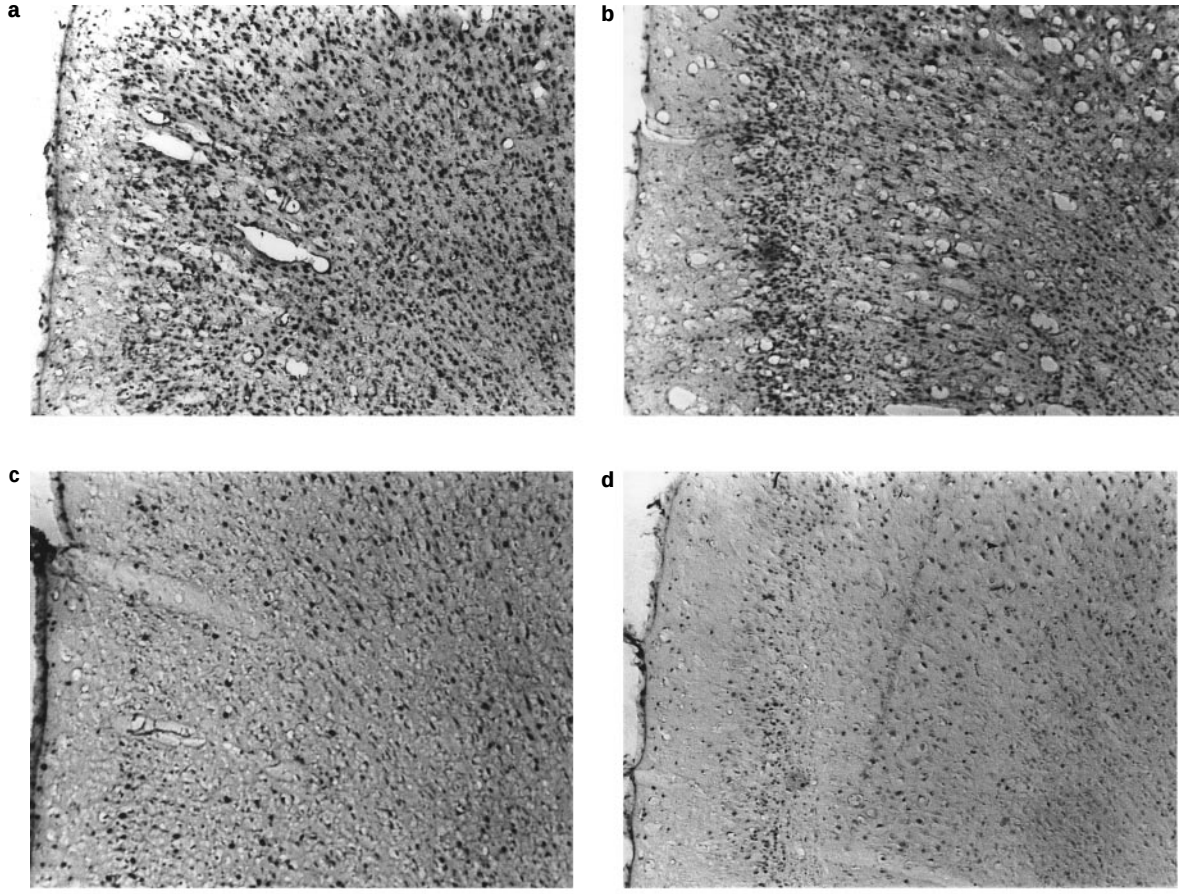

Figure 2 Effect of senktide on Fos-LI expression in the cingulate cortex of a guinea-pig treated with (a) senktide (10 nmol i.c.v. each side), (b) the less active enantiomer of the NK_3 tachykinin receptor antagonist, SR142806, 3 $mgkg^{-1}$, s.c., 30 min before senktide, (c) the NK_3 tachykinin receptor antagonist, SR142801, 3 $mgkg^{-1}$, s.c., 30 min before senktide and (d) 0.2 ml of 0.01% Tween 80, 30 min before an i.c.v. injection of 10 μl acetic acid each side. Scale bar, 80 μm.

mer, SR142806, were not significantly different from those with senktide alone and therefore most statistical comparisons have been made between results for senktide with SR142801 and senktide with SR142806. However, in the Islands of Calleja ($P=0.046$), medial amygdaloid nucleus ($P=0.009$), amygdala-hippocampal area ($P=0.025$) and posteromedian and central medial thalamic nuclei ($P=0.006$), SR142806 reduced the number of Fos-LI cells induced by senktide. In these and a few other areas comparisons have been made between senktide alone and senktide with SR142801.

In vehicle control guinea-pigs, scattered neurones throughout the cortical regions as well as the dentate gyrus, caudate putamen, lateral septum, amygdala, thalamus and hypothalamus showed detectable Fos-LI. However, these neurones were fewer in number and fainter in appearance compared to those found in brain sections of guinea-pigs treated with senktide. Control sections processed without Fos antiserum displayed virtually no staining. The greatest number of Fos-LI cells observed in control guinea-pigs was seen in the cortex, median eminence, dentate gyrus and amygdala. This is interpreted as being due to the stress of handling and injection and confirms the results of other workers which showed increased Fos-LI following stress (Sharp *et al.*, 1991).

Telencephalon

Compared with control animals, animals treated with senktide showed an increase in the number of darkly-stained Fos-LI nuclei within many telencephalic regions including the frontal, parietal, piriform and cingulate regions (Table 2). In the quantitative assessment, the frontal cortex, the cingulate cortex (Figure 2) as well as the Islands of Calleja showed increased numbers of Fos-LI neurones compared to controls (comparisons between senktide and control: $P=0.0005$ for frontal cortex 1 and 2, $P=0.015$ for the cingulate 1 region, $P<0.0001$ for islands of Calleja) (Table 3). The NK_3 tachykinin receptor antagonist, SR142801, but not its less active enantiomer, significantly reduced the number of Fos-LI cells in the cingulate cortex induced by senktide ($P=0.002$ and $P=0.0002$ for the cingulate 1 and 2 regions, respectively, for comparisons between senktide with SR142801 and senktide with SR142806 (Table 3)). Interestingly, the number of Fos-LI neurones in guinea-pigs treated with SR142801 was significantly lower than the number in control guinea-pigs ($P=0.01$ and $P=0.015$ for cingulate 1 and 2 regions for comparisons between SR142801 and control). However, SR142801 did not significantly reduce the number of Fos-LI neurones induced by senktide in the frontal cortex.

Low to moderate numbers of Fos-LI neurones were found in the nucleus accumbens, caudate putamen, globus pallidus, medial septum, bed nucleus stria terminalis, and high numbers were found in the lateral septal nucleus of senktide-treated animals (Table 2). In the lateral septal nucleus the number of Fos-LI cells in guinea-pigs treated with SR142801 was significantly reduced almost to that in control guinea-pigs ($P=0.017$ between senktide with SR142801 and senktide with SR142806) (Table 3). For the Islands of Calleja, SR142801 treatment of guinea-pigs reduced the number of Fos-LI neurones almost to control levels ($P=0.002$ for comparison between senktide alone and senktide with SR142801) (Table 3).

Senktide induced Fos-LI in neurones in the granule cell layer of the dentate gyrus (Figure 3). Both the staining intensity and number of Fos-LI cells were reduced almost to control

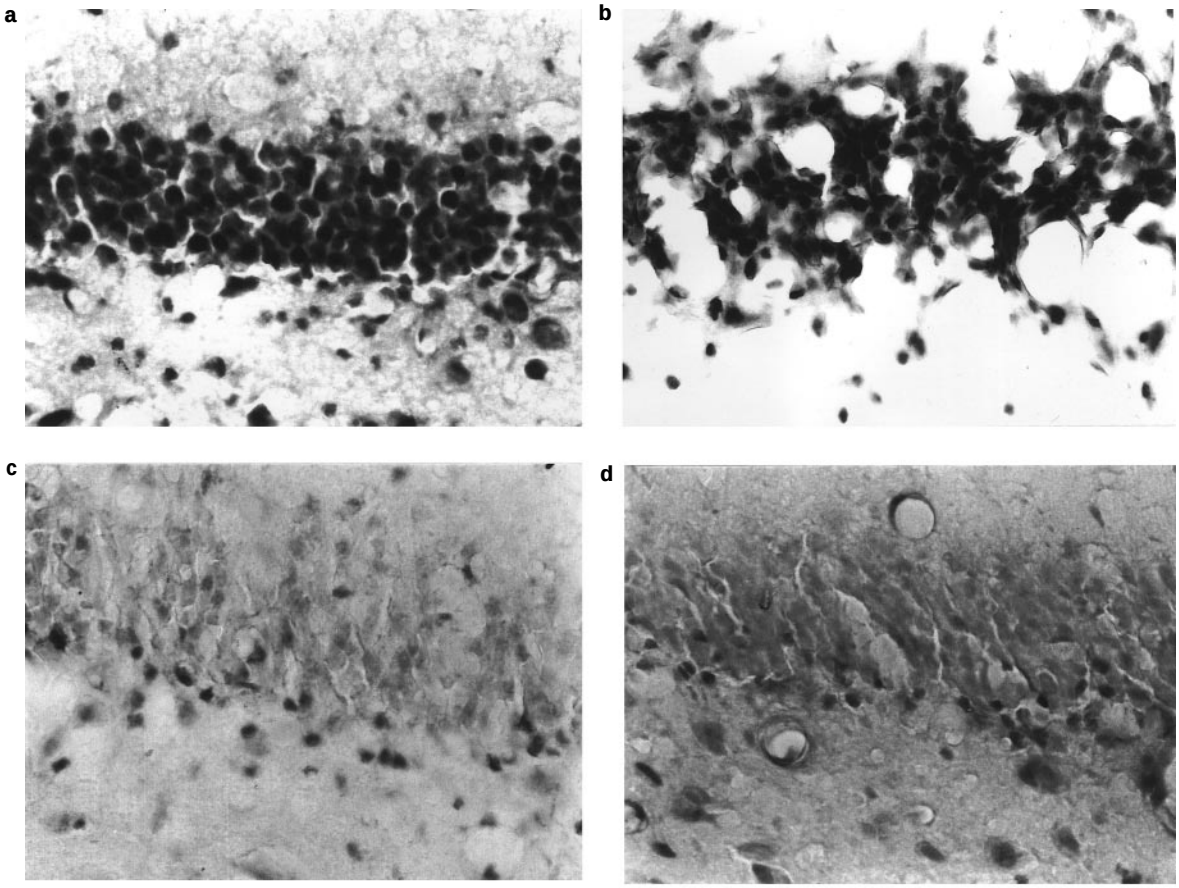

Figure 3 Neurones containing Fos-LI in the granular layer of the dentate gyrus of the hippocampal formation of guinea-pigs treated with (a) senktide (10 nmol i.c.v. each side), (b) the less active enantiomer of the NK_3 tachykinin receptor antagonist, SR142806, 3 $mgkg^{-1}$, s.c., 30 min before senktide, (c) the NK_3 tachykinin receptor antagonist, SR142801, 3 $mgkg^{-1}$, s.c., 30 min before senktide and (d) 0.2 ml of 0.01% Tween 80, 30 min before an i.c.v. injection of 10 μl acetic acid each side. Scale bar, 20 μm.

levels by treatment of guinea-pigs with SR142801 ($P=0.004$ for comparisons between senktide with SR142801 and senktide with SR142806) (Table 3). High numbers of Fos-LI cells were also observed in the amygdala in senktide-treated guinea-pigs with the most concentrated areas of staining in the medial, basomedial and basolateral amygdaloid nuclei and to a lesser extent in the posteromedial cortical amygdaloid nucleus and the amygdaloid-hippocampal area (Tables 2 and 3). Fos-LI in most areas of the amygdala was affected by treatment with SR142801. Areas affected were the posteromedian cortical amygdaloid nucleus ($P=0.011$), the basomedial ($P=0.026$) and the basolateral amygdaloid nucleus ($P=0.015$) (comparisons between senktide with SR142801 and senktide with SR142806), the medial amygdaloid nucleus ($P=0.008$) and the amygdala-hippocampal area ($P=0.046$) (comparisons between senktide alone and with SR142801). SR142801 produced a reduction in Fos-LI staining to levels not significantly different from control in the medial amygdaloid nucleus, posteromedial cortical amygdaloid nucleus and amygdala-hippocampal area, but not the basomedial or basolateral amygdaloid nucleus, where SR142801 treatment reduced the number of Fos-LI cells to below control values ($P=0.012$ and $P=0.005$, respectively, for comparison between senktide with SR142801 and control) (Table 3)

Diencephalon

Following treatment with senktide, high numbers of intensely-stained Fos-LI cells were detected in the medial part of the thalamus especially in the intermediodorsal, medial dorsal, postero median and central medial thalamic nuclei, as well as the medial and lateral habenula (Tables 2 and 3). Moderate numbers of Fos-LI stained cells were observed in the parafascicular and paraventricular thalamic nucleus, zona incerta, ventral lateral and lateral dorsal thalamic nuclei as well as medial and dorsolateral geniculate nucleus (Table 2). Senktide-induced Fos-LI in the thalamic area of the guinea-pig brain was extensively affected by treatment with SR142801. Reduction in numbers of Fos-LI neurones by SR142801 was evident in the intermediodorsal thalamic nucleus, paraventricular thalamic nucleus, posteromedian thalamic nucleus and central medial thalamic nucleus ($P=0.0004$, $P=0.021$, $P=0.0002$ for comparisons between senktide alone and with SR142801). In the medial and lateral habenula, SR142801 reduced the effect of senktide to levels not significantly different from control ($P=0.035$ for comparison between senktide with SR142801 and with SR142806) (Table 3).

In senktide-treated animals a large number of Fos-LI cells was seen in the dorsomedial and ventromedial hypothalamic nuclei and median eminence, a moderate number was detected in the periventricular and arcuate nuclei, the lateral and dorsal hypothalamic nuclei as well as the magnocellular nucleus of the lateral hypothalamus (Table 2). SR142801-treated guinea-pigs showed significant reduction in Fos-LI cells in the dorsomedial hypothalamic region ($P=0.05$ for comparison between senktide with SR142801 and with SR142806) (Table 3). However, the numbers of Fos-LI cells in the presence of SR142801 remained significantly above control levels ($P=0.015$ for comparison between senktide with SR142801 and control).

Mesencephalon

The substantia nigra, the ventral tegmental area and periaqueductal gray showed low levels of staining in both the control and senktide-treated guinea-pigs (Table 2). The numbers of Fos-LI cells observed in the interpeduncular nucleus were significantly greater in senktide-treated guinea-pigs than in controls ($P = 0.0165$) (Table 3). SR142801 was ineffective in reducing the Fos-LI staining in the interpeduncular nucleus induced by senktide (Table 3).

Metencephalon

Both the control and senktide-treated animals were found to have low levels of Fos-LI in several metencephalic regions including the pontine reticular nucleus, the lateral superior olive, the lateral parabrachial nucleus and the locus coeruleus (Table 2). However, senktide-treated animals showed intense Fos-LI compared to controls in all cerebellar lobules, presumably due to increased locomotor activity (Table 2). Low to moderate increases in Fos-LI were observed in the locus coeruleus and ventral cochlear nucleus but these did not reach statistical significance (Table 3). While the number of Fos-LI cells in the locus coeruleus was not affected by SR142801, the number in the ventral cochlear nucleus was reduced ($P = 0.017$ for comparison between senktide with SR142801 and with SR142806) (Table 3).

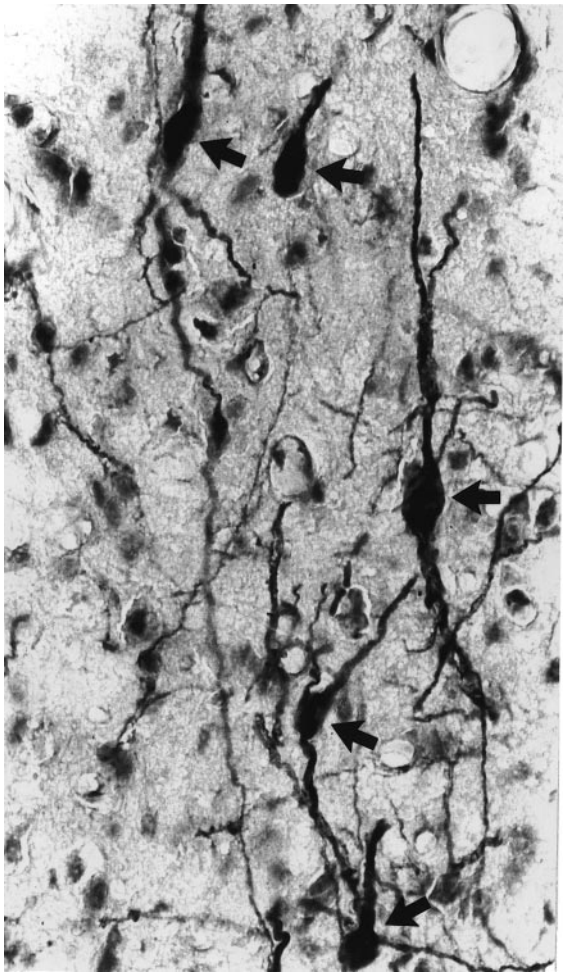

Figure 4 Neurones double-stained for Fos-LI and tyrosine-hydroxylase-LI in the hypothalamic arcuate nucleus of a guinea-pig treated with senktide (10 nmol i.c.v. each side). Arrows point to neurones double-stained with Fos-LI and tyrosine hydroxylase-LI. Scale bar, 20 μm.

Localization of Fos-LI in tyrosine hydroxylase-LI neurones

Strongly stained tyrosine hydroxylase-LI cell bodies were observed in the hypothalamic arcuate, periventricular and dorsal nuclei, paraventricular thalamic nucleus, central gray, ventral tegmental area, substantia nigra and in the linear and dorsal raphe nucleus. A few lightly-stained tyrosine hydroxylase-LI cells were also detected in the nucleus accumbens and the caudate putamen. Fos-LI was present in some tyrosine hydroxylase-LI neurones (Figure 4). Table 4 shows the mean numbers of Fos-LI negative and Fos-LI positive tyrosine hydroxylase-LI cells in particular brain regions for each treatment. In the arcuate hypothalamic nucleus, SR142801 significantly reduced the number of Fos-LI positive tyrosine hydroxylase-LI cells induced by senktide ($P = 0.0004$) (Table 4). This reduction was not reflected in a change in the number of Fos-LI negative tyrosine hydroxylase-LI neurones from the same region of the arcuate nucleus, suggesting that SR142801 did not affect the number of detectable tyrosine hydroxylase-LI neurones.

Discussion

Administration of senktide i.c.v. (10 nmol each side) produced increased locomotor activity in guinea-pigs, thus confirming the results from a previous study (Johnston & Chahl, 1993). In contrast to these findings, Piot *et al.* (1995) did not observe an increase in locomotor activity following i.c.v. administration of a lower dose of senktide (1 μg, equivalent to 0.625 nmol, each side, in the presence of phosphoramidon) to smaller guinea-pigs (145–215 g) than those used in the present study (450–550 g). Nevertheless, in agreement with previous findings (Stoessl *et al.*, 1990; Piot *et al.*, 1995), wet-dog shakes were observed in the present experiments following i.c.v. administration of senktide. Furthermore, the present experiment showed that the frequency of wet-dog shakes induced by senktide was reduced by the NK_3 tachykinin receptor antagonist, SR142801, and not by its less active enantiomer, SR142806, hence indicating a role for NK_3 tachykinin receptors in mediating wet-dog shakes. In contrast to its effect on wet-dog shakes, SR142801 did not inhibit locomotor activity induced by senktide. This result was surprising since SR142801 administered orally and intraperitoneally inhibited turning behaviour in gerbils, and, therefore, presumably crossed the blood-brain barrier (Emonds-Alt *et al.*, 1995). Furthermore, SR142801 had a long duration of action (up to 8 h in gerbils following oral administration) (Emonds-Alt *et al.*, 1995). Two possible explanations for this discrepancy are that the dose of SR142801 was inadequate, or that the dose of senktide used produced effects on other tachykinin receptors in guinea-pig brain to induce locomotor activity. It is unlikely that senktide acted on non-tachykinin receptors since the peptide tachykinin antagonist, spantide, inhibited locomotor activity induced by the dose of senktide used in the present study (Johnston & Chahl, 1993).

The immunohistochemical data in the present study show that administration of senktide i.c.v. induced Fos-LI in many areas of the guinea-pig brain, including the frontal, cingulate, parietal and piriform cortex, the lateral septum, the CA1, CA2, subiculum and dentate gyrus of the hippocampus, most areas of the amygdala, thalamus and hypothalamus, the rostral linear raphe nucleus, the cerebellum and the ventral cochlear nucleus. It would be expected that neurones bearing NK_3 tachykinin receptors and thus presumably excited by the NK_3 tachykinin receptor agonist, senktide, and also those neurones in pathways activated by such neurones would express c-Fos. One limitation of the i.c.v. route of administration is that neurones near the ventricles are likely to be exposed to a higher concentration of drug, and thus more likely to be activated directly, than more distant neurones.

Table 4 Numbers of neurones double-stained for Fos-LI and tyrosine hydroxylase in guinea-pig brain after administration of senktide

(a) Mesencephalon

	CG	*RLi*	*VTA*	*SNR*	*SNC*
Control					
TH	24.2±4.5	25.5±1.0	29.7±7.8	35.2±10.7	73.0±6.3
Fos+TH	19.5±6.0	15.2±3.3	17.5±6.1	21.5±7.7	30.7±5.6
Senktide+SR142806					
TH	33.0±6.4	35.5±4.5	25.2±5.6	91.0±20.3	93.2±13.3
Fos+TH	31.0±0.5	17.0±4.4	13.0±1.8	39.5±9.2	47.7±9.5
Senktide					
TH	22.1±2.9	30.1±5.8	21.3±3.6	51.3±10.1	47.3±7.7
Fos+TH	20.1±3.3	12.1±2.3	13.8±2.3	25.6±8.1	30.1±10.3
Senktide+SR142801					
TH	26.8±5.4	31.0±4.4	20.1±3.7	50.1±4.9	50.8±7.1
Fos+TH	16.8±4.9	14.0±1.0	8.1±2.1	24.0±5.4	31.2±3.1

(b) Diencephalon

	ARC	*Pe*	*DA*	*DMD*
Control				
TH	2.2±0.4	13.0±12.7	10.2±3.0	5.5±1.7
Fos+TH	2.5±1.8	12.7±5.5	6.2±2.0	10.2±3.0
Senktide+SR142806				
TH	0.3±0.3	16.0±3.3	0.0±0.0	4.2±2.4
Fos+TH	2.2±1.3	10.5±3.2	0.0±0.0	4.7±2.1
Senktide				
TH	4.0±1.4	13.7±2.5	6.1±1.9	8.0±1.7
Fos+TH	5.8±1.0	13.1±1.8	7.5±2.7	3.4±0.7
Senktide+SR142801				
TH	4.7±3.7	6.6±2.3	9.0±2.6	10.3±1.3
Fos+TH	0.3***±0.2	7.1*±1.3	5.0±1.2	9.8±3.7

Values are means±s.e.means of total numbers of neurones single-stained for tyrosine hydroxylase-LI (TH) and double-stained for Fos-LI and tyrosine hydroxylase-LI (Fos+TH) in (a) mesencephalic and (b) diencephalic regions of guinea-pig brain 90 min following i.c.v. injections of senktide (10 nmol each side) in untreated animals ($n=4$), and animals treated 30 min before senktide with SR142801 (3mg kg^{-1} s.c.) ($n=4$), or SR142806 (3mg kg^{-1} s.c.) ($n=4$)). Control animals were given 0.2 ml of 0.01% Tween 80 s.c. 30 min before i.c.v. injections of 10 µl each side of 20 mM acetic acid ($n=4$). Abbreviations: CG, central gray; RLi, rostral linear raphe nucleus; VTA, ventral tegmental area; SNR, substantia nigra pars reticulata; SNC, substantia nigra pars compacta; Arc, arcuate hypothalamic nucleus; Pe, periventricular hypothalamic nucleus; DA, dorsal hypothalamic nucleus; DMD, dorsomedial hypothalamic nucleus, diffuse part. The number of Fos+TH stained neurones induced by senktide in SR142801-treated animals was significantly less than in untreated animals in the arcuate nucleus, ***, $P=0.0003$, and in the periventricular nucleus, *, $P=0.024$ (Student's t tests).

The only regions of the guinea-pig brain that were shown to contain high densities of NK_3 ([^{3}H]-senktide) binding sites in autoradiographic studies, were the mid and deep cortical laminae, the medial habenula and the caudal hippocampus (Dam & Quirion, 1994). The greater distribution of Fos-LI neurones found in the present study suggests either the activation of downstream neurones, or possibly, the activation of other tachykinin receptors by senktide. For example, expression of Fos-LI in senktide-treated guinea-pigs was prominent in superficial cortical as well as middle and deep cortical laminae, possibly as a result of secondary activation via projections from deeper cortical laminae. Furthermore, moderate to high numbers of Fos-LI neurones were present following senktide administration in the striatum and some thalamic and hypothalamic areas, such as the intermediodorsal thalamic nucleus, the paraventricular nucleus of the thalamus and the dorsomedial hypothalamic nucleus, areas which were found in autoradiographic studies to have low specific labelling for [^{3}H]-senktide (Dam & Quirion, 1994). Activation of nuclei in thalamic regions by senktide might have resulted from activation by cortical areas. Although i.c.v. injection of senktide in rats causes effects on hypothalamic functions such as release of vasopressin (Takano *et al*., 1990), such functional correlates of activation of hypothalamic areas by senktide in guinea-pigs are unknown.

The results from the behavioural experiments (see above) suggested that senktide at the dose used in the present experiments might activate NK_1 tachykinin receptors. Autoradiographic localization of NK_1 binding sites with [^{3}H]-[Sar^9, Met$(O_2)^{11}$]SP in guinea-pig brain (Dam & Quirion, 1994) was not successful since [^{3}H]-[Sar^9, Met$(O_2)^{11}$]SP was a poor radioligand in guinea-pig brain. Therefore, the distribution of NK_1 binding sites in guinea-pig brain has been determined with the less selective ligand, [^{3}H]-SP, which labelled sites characterized pharmacologically as NK_1 (Dam & Quirion, 1986). Although there is extensive overlap in the distributions of NK_1 and NK_3 binding sites in the guinea-pig brain, it appears that the distribution of Fos-LI cells in senktide-treated guinea-pigs was not identical with the distribution of NK_1 binding sites. For example, Fos-LI was lower in the striatum than would be expected if senktide had activated NK_1 tachykinin receptors. However, further studies are required to elucidate the distribution of NK_1 tachykinin receptors in the guinea-pig brain.

The NK_3 tachykinin receptor antagonist, SR142801, but not its less active enantiomer, SR142806, inhibited Fos-LI expression induced by senktide in many regions of the brain, including the cingulate cortex, lateral septum, dentate gyrus, most regions of the amygdala, thalamus, habenula, dorsomedial hypothalamic nuclei and the ventral cochlear nuclei. Interestingly, in the cingulate cortex and the basomedial and basolateral amygdaloid nuclei, SR142801 reduced the number of Fos-LI cells in senktide-treated guinea-pigs to levels lower than in controls, suggesting an ongoing activation of NK_3 tachykinin receptors by endogenous tachykinins. In rats, the overall distribution of neurokinin B (NKB) was similar to that of SP (Merchenthalier *et al*., 1992), although relatively more NKB was present in the cerebral cortex (see Otsuka & Yoshioka, 1993). Therefore, it is tempting to speculate that NKB is the endogenous tachykinin involved in activation of NK_3 tachykinin receptors in this species. However, substance P is present in more cells in guinea-pig cortex and hippocampus than in rat cortex and hippocampus (Gallagher *et al*., 1992) and it is possible that substance P is the endogenous ligand at

NK_3 tachykinin receptors in guinea-pig telencephalon. SR142801 was less effective in inhibiting the effects of senktide in the frontal cortex and in hind brain regions, indicating either that senktide activated another tachykinin receptor in these regions, or that a senktide-sensitive, SR142801-resistant, NK_3 tachykinin receptor subtype is present in these regions.

Tachykinins have been proposed to play a role in learning and memory possibly by a modulatory action in the hippocampus and amygdala (Flood *et al*., 1990; Nagel *et al*., 1993). The results from the present study have shown that senktide activated many limbic regions including the hippocampus and amygdala by acting on NK_3 tachykinin receptors, supporting the involvement of these receptors in learning and memory.

A novel finding from the present study was the induction of Fos-LI in the ventral cochlear nucleus by senktide, an effect that was inhibited by SR142801. In the ventral cochlear nucleus, activation of Fos-LI has been associated with certain low tone acoustic stimulation (Sato *et al*., 1992; Eybalin, 1993). Further investigations are required to determine the role of NK_3 tachykinin receptors in auditory perception.

The distribution of tyrosine hydroxylase-LI in the guinea-pig brain was similar to that described for rats (Hökfelt *et al*., 1984). Senktide administration induced Fos-LI in tyrosine hydroxylase-LI neurones of the arcuate hypothalamic nucleus and this effect was significantly inhibited by SR142801. Dopamine-containing neurones are known to be present in the arcuate nucleus (Björklund & Lindvall, 1984). Tachykinin-containing nerve terminals have been shown to make contact with tyrosine hydroxylase neurones in the arcuate nucleus in rats (Magoul *et al*., 1993). Although similar studies have not been performed in guinea-pigs, the results from the present study would support the proposal that activation of NK_3 tachykinin receptors releases dopamine from the arcuate nucleus in guinea-pigs. Dopamine has been implicated in the actions of tachykinins in other regions, particularly in the nigrostriatal system and ventral tegmental area (see Kalivas, 1993; Otsuka & Yoshioka, 1993). However, in the present study senktide failed to induce an increase in the number of Fos-positive, tyrosine hydroxylase-LI neurones in the substantia nigra, an area which exhibited the most intense tyrosine hydroxylase staining. Although in rats Keegan *et al*. (1992) found an excitatory response to senktide in a subpopulation of dopamine-sensitive neurones in the substantia nigra, the lack of effect of senktide in the substantia nigra in the present study was not unexpected since guinea-pigs, unlike rats, do not have NK_3 tachykinin receptors in this region (Dam & Quirion, 1994). For several years the substantia nigra has been considered to be a striking example of mismatch between transmitter and receptor localization. Recently the substantia nigra in the rat has been shown to contain NK_3 tachykinin receptors (Dam & Quirion, 1994; Ding *et al*., 1996) which may be involved in the action of tachykinins in this region. However, the nature of the tachykinin receptor present in guinea-pig substantia nigra remains unknown.

In conclusion, the present study has added to current knowledge obtained from receptor localization studies, by providing a functional map of the neuronal populations activated directly or indirectly by the NK_3 tachykinin receptor agonist, senktide, in guinea-pig brain. The availability of an NK_3 tachykinin receptor antagonist has revealed that activation of neurones in the cortex, hippocampus, thalamus, hypothalamus, amygdala and ventral cochlear nuclei by senktide is indeed mediated by NK_3 tachykinin receptors. Thus NK_3 tachykinin receptors may play a more extensive role in the control of diverse brain functions including cortical processing, learning and memory, neuroendocrine and behavioural regulation than is currently recognized. This knowledge should further studies on the physiological role of NK_3 tachykinin receptors in the CNS.

This research was supported by the National Health and Medical Research Council of Australia. The donation of SR142801 and SR142806 by Dr Emonds-Alt from Sanofi Recherche is gratefully acknowledged. We wish to thank Mrs Ann Lynch-Frame for technical assistance.

References

BJÖRKLUND, A. & LINDVALL, O. (1984). Dopamine-containing system in the CNS. In *Handbook of Chemical Neuroanatomy*, Vol. **2,** *Classical Transmitters in the CNS*. ed. Björklund, A. & Hökfelt, T. Part 1. pp. 55–111, Amsterdam: Elsevier.

BOT, G. & CHAHL, L.A. (1996). Induction of Fos-like immunoreactivity by opioids in guinea-pig brain. *Brain Res*., **731,** 45–56.

BOT, G., CHAHL, L.A., BRENT, P.J. & JOHNSTON, P.A. (1992). Effects of intracerebroventricularly administered mu-, delta- and kappa-opioid agonists in locomotor activity of the guinea-pig and the pharmacology of the locomotor response to U50, 488H, *Neuropharmacology*, **31,** 825–833.

DAM, T.V. & QUIRION, R. (1994). Comparative distribution of receptor types in the mammalian brain, In *The Tachykinin Receptors*. ed. Buck, S.H., pp. 101–123. New Jersey: Humana Press.

DAM, T.V. & QUIRION, R. (1986). Pharmacological characterization and autoradiographic localization of substance P receptors in guinea pig brain, *Peptides*, **7,** 855–864.

DING, Y.Q., SHIGEMOTO, R., TAKADA, M., OHISHI, H., NAKANISHI, S. & MIZUNO, N. (1996). Localization of neuromedin K receptor (NK_3) in the central nervous system of the rat. *J. Comp. Neurol*., **364,** 290–310.

ELLIOTT, P.J. & IVERSEN, S.D. (1986). Behavioural effects of tachykinins and related peptides. *Brain Res*., **381,** 68–76.

EMONDS-ALT, X., BICHON, D., DUCOUX, J.P., HEAULME, M., MILOUX, B., PONCELET, M., PROIETTO, V., VAN BROECK, D., VILAIN, P., NELIAT, G., SOUBRIE, P., LE FUR, G. & BRELIERE, J.C. (1995). SR142801, the first potent non-peptide antagonist of the tachykinin NK_3 receptor. *Pharmacol. Letts*, **56,** 27–32.

EYBALIN, M. (1993). Neurotransmitters and neuromodulators of the mammalian cochlear. *Physiol. Rev*., **73,** 309–353.

FARDIN, V. & GARRET, C. (1991). Species differences between [^{3}H] substance P binding in the rat and guinea-pig shown by the use of peptide agonists and antagonists. *Eur. J. Pharmacol*., **210,** 231–234.

FLOOD, J.F., BAKER, M.L., HERNANDEZ, E.N. & MORLEY, J.E. (1990). Modulation of memory retention by neuropeptide K. *Brain Res*., **520,** 284–290.

GALLAGHER, A.W., CHAHL, L.A. & LYNCH, A.M. (1992). Distribution of SP-like immunoreactivity in guinea-pig central nervous system. *Brain Res. Bull*., **29,** 199–207.

HAI, T. & CURRAN, T. (1991). Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. *Proc. Natl. Acad. Sci. U.S.A*., **88,** 3720–3724.

HÖKFELT, T., MARTENSSON, R., BJÖRKLUND, A., KLEINAU, S., BJÖRKLUND, A. & GOLDSTEIN, M. (1984). Distribution maps of tyrosine-hydroxylase-immunoreactive neurons in rat brain. In *Handbook of Chemical Neuroanatomy*, Vol **2,** *Classical Transmitters in the CNS*. ed. Björklund, A. & Hökfelt, T. Part 1. pp. 277–379. Amsterdam: Elsevier.

HSU, S.M. & SOBAN, E. (1982). Colour modification of diaminobenzidine (DAB) precipitation by metallic ions and its application for double immunohistochemistry. *J. Histochem. Cytochem*., **30,** 1079–1082.

JOHNSTON, P.A. & CHAHL, L.A. (1991). Tachykinin antagonists inhibit the morphine withdrawal response in guinea-pigs. *Naunyn-Schmiedeberg's Arch. Pharmacol*., **343,** 283–288.

JOHNSTON, P.A. & CHAHL, L.A. (1993). Neurokinin-1, -2 and -3 receptors are candidates for a role in the opiate withdrawal response in guinea-pigs. *Regul. Peptides*, **46,** 376–378.

KALIVAS, P.W. (1993). Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res. Rev.*, **18,** 75–113.

KEEGAN, K.D., WOODRUFF, G.N. & PINNOCK, R.D. (1992). The selective NK_3 receptor agonist senktide excites a subpopulation of dopamine-sensitive neurones in the rat substantia nigra pars compacta *in vitro*. *Br. J. Pharmacol.*, **105,** 3–5.

MAGGI, C.A. (1995). The mammalian tachykinin receptors, *Gen. Pharmacol.*, **26,** 911–944.

MAGOUL, R., DUBORG, P., BENJELLOUN, W. & TRAMU, G. (1993). Synaptic inputs of tachykinin-containing nerve terminals to target tyrosine-hydroxylase-, β-endorphin- and neuropeptide Y-producing neurons of the arcuate nucleus. Double pre-embedding immunocytochemical study in the rat. *J. Chem. Neuroanat.*, **6,** 419–429.

MARKSTEINER, J., SPERK, G. & KRAUSE, J.E. (1992). Distribution of neurons expressing neurokinin B in the rat brain: immunohistochemistry and in situ hybridization. *J. Comp. Neurol.*, **317,** 341–356.

MERCHENTHALIER, I., MADERDRUT, J.L., O'HARTE, F. & CONLON, J.M. (1992). Localization of neurokinin B in the central nervous system of the rat. *Peptides*, **13,** 815–819.

MORGAN, J.I. & CURRAN, T. (1991). Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. *Ann. Rev. Neurosci.*, **14,** 421–452.

NAGEL, J.A., WELZL, H., BÄTTIG, K. & HUSTON, J.P. (1993). Facilitation of tunnel maze performance by systemic injection of the neurokinin substance P. *Peptides*, **14,** 85–95.

NAKANISHI, S. (1991). Mammalian tachykinin receptors. *Annu. Rev. Neurosci.*, **14,** 123–136.

OTSUKA, M. & YOSHIOKA, K. (1993). Neurotransmitter functions of mammalian tachykinins. *Physiol. Rev.*, **73,** 229–308.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereostaxic Coordinates*. 2nd ed. Sydney: Academic Press.

PIOT, O., BETSCHART, J., GRALL, I., RAVARD, S., GARRET, C. & BLANCHARD, J-C. (1995). Comparative behavioural profile of centrally administered tachykinin NK_1, NK_2 and NK_3 receptor agonists in the guinea-pig. *Br. J. Pharmacol.*, **116,** 2496–2502.

REGOLI, D., BOUDON, A. & FAUCHERE, J.L. (1994). Receptors and antagonists for substance P and related peptides. *Pharmacol. Rev.*, **46,** 551–599.

SATO, K., HOUTANI, T., UEYAMA, T., IKEDA, M., YAMASHITA, T., KUMAZAWA, T. & SUGIMOTO, T. (1992). Mapping of the cochlear nucleus subregions in the rat with neuronal protein induced by acoustic stimulation with low tones. *Neurosci. Letts*, **142,** 48–52.

SHARP, F.R., SAGER, S.M., HICKS, K., LOWENSTEIN, D. & HISANAGA, K. (1991). c-fos mRNA, Fos and Fos-related antigen induction by hypertonic saline and stress. *J. Neurosci.*, **11,** 2321–2331.

STOESSL, A.J., DOURISH, C.T. & IVERSEN, S.D. (1988). The NK-3 tachykinin receptor agonist senktide elicits 5-HT-mediated behaviour following central or peripheral administration in mice and rats. *Br. J. Pharmacol.*, **94,** 285–287.

STOESSL, A.J., DOURISH, C.T. & IVERSEN, S.D. (1990). Pharmacological characterization of the behavioural syndrome induced by the NK-3 tachykinin agonist senktide in rodents: evidence for mediation by endogenous 5-HT. *Brain Res.*, **517,** 111–116.

SUMAN-CHAUHAN, C.N., GRIMSON, P., GUARD, S., MADDEN, Z., CHUNG, F.Z., WATLING, K., PINNOCK, R. & WOODRUFF, G. (1994). Characterisation of [^{125}I][MePhe^7] neurokinin B binding to tachykinin NK_3 receptors: evidence for interspecies variance. *Eur. J. Pharmacol.*, **269,** 65–72.

TAKANO, Y., NAGASHIMA, A., HAGIO, T., TATEISHI, K. & KAMIYA, H. (1990). Role of central tachykinin peptides in cardiovascular regulation in rats. *Brain Res.*, **528,** 231–237.

(Received September 5, 1996
Revised April 1, 1997
Accepted July 7, 1997)