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The substance P(1–7) fragment is a potent modulator of substance P actions in the brain

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The neuropeptide, substance P (SP), produces a spectrum of behavioural effects. When given locally into the substantia nigra, SP induces dopamine release in the ipsilateral striatum and produces contralateral rotation in a dose-dependent, but bell-shaped, manner. Similar dose–response relationships have been observed for SP and other peptides in different bioassays. To test whether SP fragmentation is responsible for this phenomenon, SP(1–7), which is the main SP fragment in rat CNS, was injected intranigrally. SP(1–7) was found to act as a very potent antagonist against the SP-induced responses and was formed locally in the nigra after SP injection. It is proposed that SP(1–7) is an endogenous modulator of SP actions. Generation of peptide fragments, which retain receptor affinity but not efficacy, may be a general mechanism for autoregulation in peptidergic systems.

Substance P (SP) has very complex actions if administered into the rat CNS[9,16,19,21]. Some of these actions can be amplified and sustained by the use of analogs which are metabolically more resistant[7], indicating the importance of metabolic degradation.

The striatonigral SP projection in rat brain is a major pathway[4,10,14,17] and SP is very potent in producing contrateral rotation if injected into the substantia nigra[16,18,21]. It has been shown that this response is dependent upon an intact dopaminergic striatonigral pathway[12,16] and indeed, in the striatum, dopamine (DA) release is increased following nigral SP administration[6,12,24]. These responses, however, show anomalous dose–response curves with lower responses at higher doses[12,24]. It occurred to us that this might be due to the generation of SP fragments which modulate SP action.

Of several possible fragments, SP(1–7) was chosen for study, because it is the major fragment in rat CNS[26] and may produce behavioural responses which are different to those produced by the parent compound[11]. In a separate paper[25] the interaction between substance P and the N-terminal and C-terminal fragments is further studied. The SP(1–7) fragment was administered intranigrally alone and in combination with SP. The effects on rotational behaviour[13,29] and on DA release monitored with microdialysis[30] were studied. SP(1–7) was found to potently antagonize the effects of SP on striatal DA release and rotational behaviour. Following intranigral SP administration, SP(1–7) was found to be formed from SP in the injected substantia nigra.

Sprague–Dawley male rats weighing 250–300 g were anaesthetized with halothane and placed in a stereotaxic frame. An injection cannula, conically shaped with a penetration tip diameter of approximately 0.15 mm was lowered into the SNR (coordinates: B 6.0, L –2.0, V 8.5, according to the Paxinos and Watson atlas[22]). Saline (0.2 μl), SP (0.007–0.7 nmol) or SP(1–7) (0.01–1 nmol) was injected into the left substantia nigra, pars reticulata (SNR) and the rat was placed in a rotometer[29]. The substances were injected in a total volume of 0.2 μl over a period of 1 min. A group of animals was sacrificed by decapitation 1 hour after the injection, their brains were immediately removed and tissue samples were taken from left and right striatum, globus pallidum (GP) and substantia nigra (SN). Samples were assayed for SP and SP(1–7). Tissue samples were extracted with hot 1 M acetic acid, lyophilized and subjected to ion exchange separation. Fractions containing SP and SP(1–7) were isolated and measured in specific RIA's[26]. The sensitivity of the assays were 2.5 fmol/tube SP and 10 fmol/tube SP(1–7), respectively. Another group of rats was used for microdialysis experiments. A microdialysis probe (CMA 10, Carnegie Medicin, Stockholm, Sweden) was implanted in the left striatum, (coordinates: B 1.2, L –2.4, V –7.5). The microdialysis probe was perfused with Ringer (in mM: 147 Na^+, 2.3 Ca^{2+}, 4 K^+ and 155.6 Cl^-,

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TABLE I

Rotational behaviour produced by substance P (SP) and SP 1–7 injected into the left substantia nigra, pars reticulata (volume of injections = 0.2 μl)

Maximal values for SP are indicated in italics.

Treatment doses (nmol/μl)	*N*	*Ipsilateral rotation*		*Contralateral rotation*		*Duration (min)*
		Total (turns)	*Maximum intensity (turns per 10 min)*	*Total (turns)*	*Maximum intensity (turns per 10 min)*	
Saline (0.2 μl)	*8*	*25 ± 17*	*10 ± 2*	*32 ± 11*[b]	*9 ± 3*	*60*
SP						
0.00007	5	64 ± 21	5 ± 2	224 ± 40	19 ± 7	>180
0.0007	5	97 ± 38	3 ± 2	563 ± 70	46 ± 11	>250
0.007	*11*	*44 ± 8*	*6 ± 1*	*641 ± 41*	*49 ± 1*	*>250*
0.07	4	34 ± 10	6 ± 3	230 ± 18	40 ± 6	>250
0.7	4	9 ± 3	2 ± 2	208 ± 32	36 ± 4	>250
SP(1–7)						
0.01	5	14 ± 7	3 ± 1	107 ± 22	7 ± 2	>90
0.1	7	31 ± 15	3 ± 1	127 ± 16	16 ± 4	>90
1.0	6	14 ± 11	2 ± 2	418 ± 39	32 ± 5	>200
SP + SP(1–7)						
0.007 + 0.1	4	5 ± 2	2 ± 2	355 ± 89[a]	27 ± 11[a]	>200
0.007 + 0.01	5	128 ± 24	18 ± 4	115 ± 30[a,b]	19 ± 4[a,b]	>200

[a] $P < 0.05$, as compared to effect produced by 0.007 nmol of SP alone.
[b] n.s. as compared to ipsilateral counts, which in terms of the rotational model means no rotation[16].

pH adjusted to approximately 7 by de-gassing with helium) at a constant flow of 2 μl/min. Under continued halothane anaesthesia (maintained by free breathing of 1–1.5% of halothane kept by an air flow of 1.5 l/min into a mask fitted over the nose of the rat), perfusates were collected every 20 min and then injected directly onto a high-performance liquid chromatography (HPLC) coupled to an electrochemical detector for the measurement of DA[24]. After the levels of DA stabilized, an injection cannula was placed into the left SNR and an additional 20 min perfusate fraction was collected. Thereafter, drug or saline was injected. At least 8 more 20 min fractions were collected before the microdialysis experiment was finished.

Table I shows the rotational response to intranigral injections of graded doses of SP or SP(1–7). SP was clearly more potent with a maximum at 0.007 nmol (higher doses of SP produced a lower response), whereas SP(1–7) produced a maximal response at the dose of 1.0 nmol. The rotation induced by 0.007 nmol SP was inhibited by 0.1 nmol of SP(1–7) and blocked by 0.01 nmol of SP(1–7). A similar result was observed when measuring DA release (Table II). SP at 0.07 nmol increased DA release[24] whereas 0.01 nmol SP(1–7)

TABLE II

Effects of substance P (SP) and SP (1–7) injections into the left substantia nigra, pars reticulata, on dopamine levels measured in 20 min perfusates collected from the ipsilateral striatum by microdialysis in halothane-anaesthetized rats

Changes are expressed as the percentage of the level detected at the –20–0 min period when the injection cannula was implanted into the substantia nigra (basal dopamine level = 6 ± 2 nM, $N = 14$).

Treatment dose (nmol/μl)	*N*	*Perfusion period (min)*							
		–20–0	*0–20*	*20–40*	*40–60*	*60–80*	*80–100*	*100–120*	*120–140*
Saline (0.2 μl)	9	100	100 ± 4	102 ± 5	103 ± 9	103 ± 9	95 ± 10	96 ± 10	89 ± 10
SP 0.07	8	100	119 ± 8	134 ± 14	147 ± 17**	143 ± 14*	153 ± 17**	149 ± 14**	141 ± 12*
SP (1–7) 0.01	4	100	89 ± 4	83 ± 6*	82 ± 7*	77 ± 7*	81 ± 6*	83 ± 7	83 ± 5*
SP + SP (1–7) 0.07 + 0.01	4	100	97 ± 5	94 ± 4	94 ± 4	86 ± 6	83 ± 7	85 ± 5*	83 ± 5*

*$P < 0.05$.
**$P < 0.01$.

significantly decreased the DA release. In combination, SP(1–7) completely blocked the response to SP. We have recently found that C-terminal fragments of SP tested at the same dose range with the same protocol do not modify the SP response[25]. In saline-injected animals, the regional distribution of SP(1–7) was found to be parallel to that of SP (substantia nigra > globus pallidum > striatum) (Fig. 1). SP(1–7) levels were, however, only about 1/15 of SP levels. We tested whether SP(1–7) would increase following administration of SP. We found that after intranigral injection of 0.7 nmol of SP, both SP and SP(1–7) increased in the injected substantia nigra but not in other regions (Fig. 2). After intranigral injection of 1 nmol of SP(1–7), only SP(1–7) increased in the substantia nigra (Fig. 3). Thus, the levels of SP(1–7) were increased in parallel with the increase in SP, but not vice versa, indicating that SP(1–7) must derive from the injected SP. It is suggested therefore that SP(1–7) formation might be responsible for the bell-shaped dose–response curves observed after nigral administra-

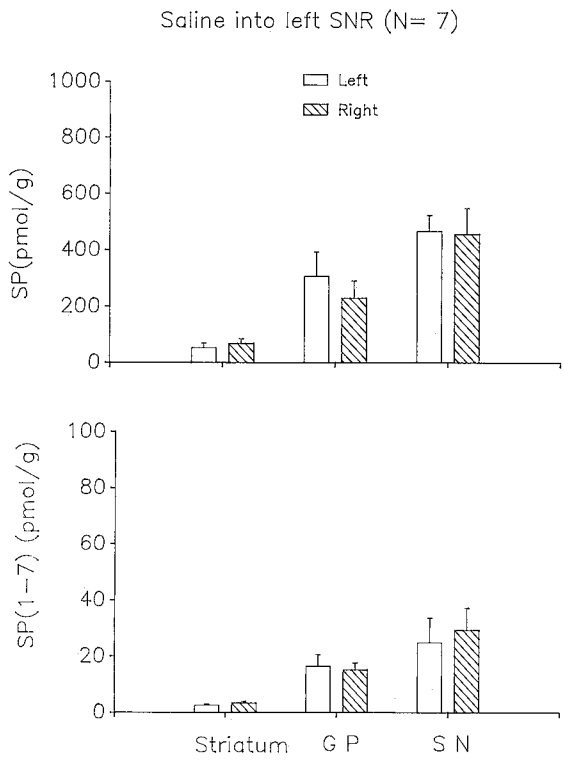

Fig. 1. Substance P (SP) and SP (1–7) levels (pmol/g) in striatum, globus pallidum (GP) and substantia nigra (SN) of rats injected with 0.2 μl saline into the left substantia nigra, pars reticulata (SNR). One h after the injection the animals were sacrificed by decapitation, the brain was immediately removed, placed on the stage of a Leitz cryomicrotome and frozen with CO_2. Serial sections were then made and samples from the right and left striatum, GP and SN were taken using 3 or 2 mm steel punches, respectively. The samples were then stored in previously weighed Eppendorf tubes at −80 °C until assayed for SP and SP(1–7). Vertical lines show S.E.M. Significant differences from control are indicated by asterisks ($P < 0.01$ for one-tailed, Student's t-test).

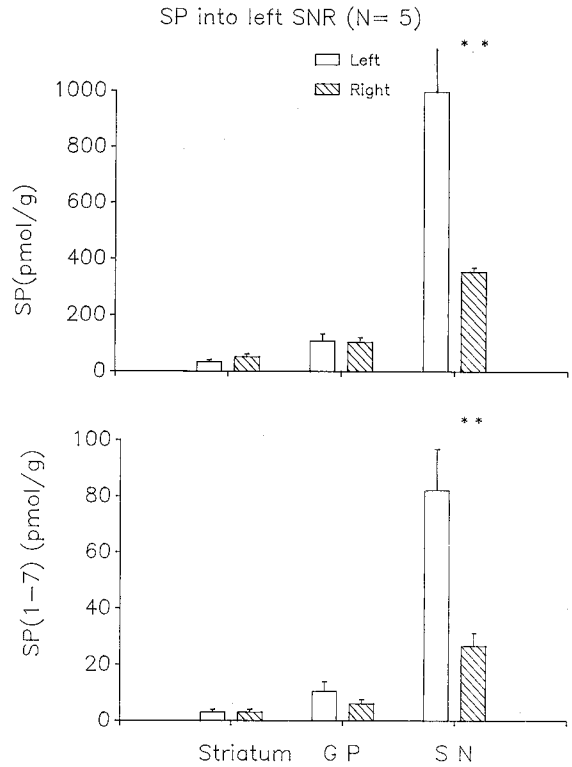

Fig. 2. SP and SP (1–7) levels (pmol/g) in striatum, GP and SN of rats injected with 0.7 nmol SP into the left SNR (see legend to Fig. 1).

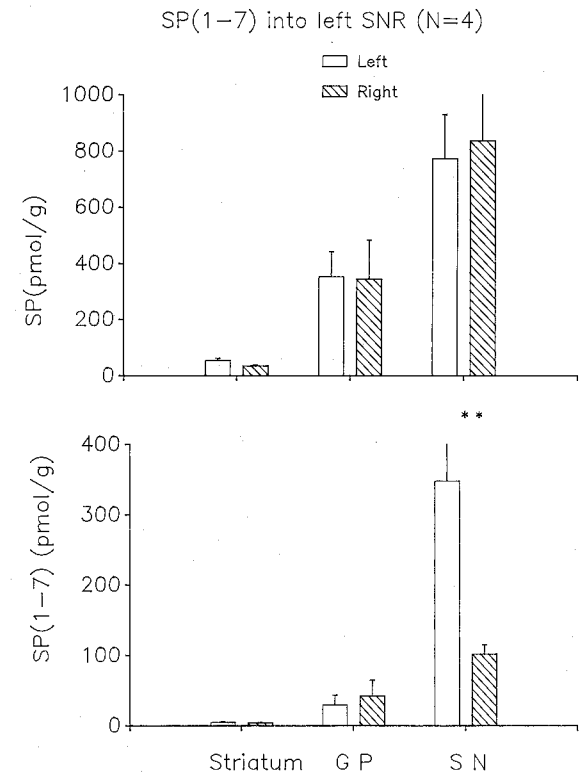

Fig. 3. SP and SP (1–7) levels (pmol/g) in striatum, GP and SN of rats injected with 1 nmol SP (1–7) into the left SNR (see legend to Fig. 1).

tion of SP. Thus SP(1–7) might be an endogenous modulator of the SP action. It remains to be elucidated at which site SP and SP(1–7) interact, since, at present, the existence of tachykinin receptors in the substantia nigra has not been conclusively demonstrated with radioactive tracer technique (for a discussion, see ref. 25).

A survey of the peptide pharmacology literature, reveals several analogous observations. As for SP[11,19], bell-shaped dose–response curves have been observed for the nonapeptide FTS[1], β-endorphin[27], VIP[2] and gp120, an HIV-enfolded protein[3]. Fragments of β-endorphin have been reported to have antagonistic properties[20,23]. Indeed, β-endorphin (1–27) antagonizes β-endorphin analgesia, but not analgesia induced by morphine, a μ receptor agonist, D-Pen^2-D-Pen^5-enkephalin, a δ receptor agonist, or U50,488H, a κ receptor agonist[28]. It has been postulated that the interaction between small, conformationally flexible peptides and their receptors occurs by a 'zipper' mechanism involving consecutive conformational rearrangements of the peptide[5]. It is conceivable that the energy gained by the complex formation is higher in certain regions of the 'zipper' than in others. This could explain why SP(1–7) but not C-terminal fragments can act as antagonist[25].

The present results might further characterize differences between 'classical' and peptide neurotransmission. The release and extracellular levels of 'classical' neurotransmitters, like monoamines are largely regulated by the presence of autoreceptors[8] and presynaptic 'uptake' sites[15], features which have not yet been described for peptides. Regulation of peptide action may, beside metabolic inactivation, occur via enzymatic conversion to fragments with antagonistic properties. The synthesis and testing of partial peptide sequences for antagonist activity may become a general principle in the development of selective peptide antagonists and better define the peptide–receptor interactions.

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1 Brand, A., Gilmour, D.G. and Goldstein, G., Effects of a nonapeptide FTS on lymphocyte differentiation in vitro, *Nature (Lond.)*, 269 (1977) 597–598.
2 Brenneman, D.E. and Eiden, L.E., Vasoactive intestinal peptide and electrical activity influence neuronal survival, *Proc. Natl. Acad. Sci. U.S.A.*, 83 (1986) 1159–1162.
3 Brenneman, D.E., Westbrook, G.L., Fitzgerald, S.P., Ennist, D.L., Elkins, K.L., Ruff, M.R. and Pert, C.B., Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide, *Nature (Lond.)*, 335 (1988) 639–642.
4 Brownstein, M.J., Mroz, E.A., Tappaz, M.L. and Leeman, S.E., On the origin of substance P and glutamic decarboxylase (GAD) in the substantia nigra, *Brain Research*, 135 (1977) 315–323.
5 Burgen, A.S.V., Roberts, G.C.K. and Fenney, J., Binding of flexible ligands to macromolecules, *Nature (Lond.)*, 253 (1975) 753–755.
6 Cheramy, A., Nieoullon, A., Michelot, R. and Glowinski, J., Effects of intranigral application of dopamine and substance P on the in vivo release of newly synthesized [^{3}H]dopamine in the ipsilateral caudate nucleus of the rat, *Neurosci. Lett.*, 4 (1977) 105–109.
7 Eison, A.S., Iversen, S.D., Sandberg, B.E.B., Watson, S.P., Hanley, M.R. and Iversen, L.L., Substance P analogue, DiME-C7: evidence for stability in rat brain and prolonged central actions, *Science*, 215 (1982) 188–190.
8 Farnebo, L.-O. and Hamberger, B., Drug-induced changes in the release of [^{3}H]monoamines from field stimulated rat brain slices, *Acta Physiol. Scand. Suppl.*, 371 (1971) 35–44.
9 Frederickson, R.C., Burgis, V., Harrel, E. and Edwards, J., Dual actions of substance P on nociception: possible role of endogenous opioids, *Science*, 199 (1978) 1359–1362.
10 Gale, K., Hong, J.S. and Guidotti, A., Presence of substance P and GABA in separate striatonigral neurons, *Brain Research*, 136 (1977) 371–375.
11 Hall, M.E. and Stewart, J.M., Substance P and behavior: opposite effects on N-terminal and C-terminal fragments, *Peptides*, 4 (1983) 763–768.
12 Herrera-Marschitz, M., Christensson-Nylander, I., Sharp, T., Staines, W., Reid, M., Hökfelt, T., Terenius, L. and Ungerstedt, U., Striato-nigral dynorphin and substance P pathways in the rat brain. II. Functional analysis, *Exp. Brain Res.*, 64 (1986) 193–207.
13 Herrera-Marschitz, M. and Ungerstedt, U., Evidence that striatal efferents relate to different dopamine receptors, *Brain Research*, 323 (1984) 269–278.
14 Hong, J.S., Yang, H.Y., Racagni, G. and Costa, E., Projections of substance P containing neurons from neostriatum to substantia nigra, *Brain Research*, 122 (1977) 541–544.
15 Iversen, L.L., Catecholamine uptake processes, *Br. Med. Bull.*, 29 (1972) 130–135.
16 James, T.A. and Starr, M.S., Behavioural and biochemical effects of substance P injected into the substantia nigra of the rat, *J. Pharm. Pharmacol.*, 29 (1977) 18–182.
17 Kanazawa, I., Emson, P.C. and Cuello, C., Evidence for the existence of substance P containing fibers in striato-nigral and pallido-nigral pathways in rat brain, *Brain Research*, 119 (1977) 447–453.
18 Kelley, A.E. and Iversen S.D., Substance P infusion into substantia nigra of rat: behavioural analysis and involvement of striatal dopamine, *Eur. J. Pharmacol.*, 60 (1979) 171–179.
19 Krivoy, W.A., Couch, J., Stewart, M.J. and Zimmermann, E., Modulation of cat monosynaptic reflexes by substance P, *Brain Research*, 202 (1980) 365–372.
20 Lee, N.M., Friedman, H.J., Leybin, L., Cho, T.M., Loh, H.H. and Li, C.H., Peptide inhibition of morphine- and β-endorphin-induced analgesia, *Proc. Natl. Acad. Sci. U.S.A.*, 77 (1980) 5525–5526.
21 Olpe, H.R. and Koella, W.P., Rotatory behaviour in rats by intranigral application of substance P and eledoisin fragment, *Brain Research*, 126 (1977) 576–579.
22 Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic Press, New York, 1982.
23 Petraglia, F., Vale, W. and Rivier, C., Opioids act centrally to modulate stress-induced decrease in luteinizing hormone in the rat, *Endocrinology*, 119 (1986) 2445–2450.
24 Reid, M., Herrera-Marschitz, M., Hökfelt, T., Terenius, L. and Ungerstedt, U., Differential modulation of striatal dopamine release by intranigral injection of gamma-aminobutyric acid (GABA), dynorphin A and substance P, *Eur. J. Pharmacol.*, 147 (1988) 411–420.

25 Reid, S.M., Herrera-Marschitz, M., Terenius, L. and Ungerstedt, U., Intranigral substance P modulation of striatal dopamine: interaction with N-terminal and C-terminal substance P fragments, *Brain Research*, in press.
26 Sakurada, T., Le Greves, P., Stewart, J. and Terenius, L., Measurement of substance P metabolites in rat CNS, *J. Neurochem.*, 44 (1985) 718–722.
27 Shanker, G. and Sharma, R.K., β-Endorphin stimulated corticosterone synthesis in isolated rat adrenal cells, *Biochem. Biophys. Res. Commun.*, 86 (1979) 1–5.
28 Suh, H.H., Tseng, L.F. and Li, C.H., β-Endorphin-(1-27) antagonizes β-endorphin — but not morphine — D-Pen^2-D-Pen^5-enkephalin- and U50,488H-induced analgesia in mice, *Neuropharmacology*, 27 (1988) 957–963.
29 Ungerstedt, U. and Arbuthnott, G., Quantitative recording of rotational behaviour in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system, *Brain Research*, 24 (1970) 485–493.
30 Ungerstedt, U., Herrera-Marschitz, M., Jungnelius, U., Ståhle, L., Tossman, U. and Zetterström, T., Dopamine synaptic mechanisms reflected in studies combining behavioral recordings and brain dialysis. In M. Kohsaka, T. Shomori, Y. Tsukada and G.N. Woodruff (Eds.), *Advances in Dopamine Research, Advances in the Biosciences, Vol. 37*, Pergamon Press, Oxford, 1982, pp. 219–231.