Pharmacology Biochemistry & Behavior, Vol. 41, pp. 75–78. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/92 $5.00 + .00

The Substance P Fragment SP(1–7) Stimulates Motor Behavior and Nigral Dopamine Release

MICHAEL E. HALL[1] AND JOHN M. STEWART

Department of Biochemistry and Neuroscience Training Program
University of Colorado Health Sciences Center, Denver, CO 80262

Received 1 July 1991

HALL, M. E. AND J. M. STEWART. *The substance P fragment SP(1–7) stimulates motor behavior and nigral dopamine release.* PHARMACOL BIOCHEM BEHAV **41**(1) 75–78, 1992.—Earlier studies have shown that the undecapeptide substance P (SP) alters motor behavior and dopamine metabolism following injection into the substantia nigra (SN) in rat, even though the SN appears largely devoid of SP-specific (NK-1) receptors. In this report, intra-nigral injections of the amino-terminal SP fragment SP(1–7) enhanced rearing, sniffing and locomotor activity, and increased the nigral DOPAC-to-DA ratio. In addition, SP(1–7) increased ^{3}H-DA release from the SN in vitro. These findings suggest that some of the effects of nigral SP on motor behavior and dopamine release are mediated by amino-terminal fragments of SP.

Substance P SP(1–7) Substantia nigra Motor behavior Dopamine

THE peptide neurotransmitter substance P (SP) is widely distributed within the mammalian nervous system (1). SP is a member of the tachykinin family of peptides, all of which share a similar carboxy-terminal sequence that is both necessary and sufficient for tachykinin-like activity (6). As with other peptide transmitters, the actions of SP are thought to be terminated by enzymatic cleavage (12). Several peptidases have been shown capable of cleaving SP, and various metabolites have been demonstrated in vivo. The most common metabolites in CNS are the N-terminal fragments SP(1–6), SP(1–7) and SP(1–9), which are produced by the action of peptidases such as endopeptidase 3.4.24.11 (15, 16, 24).

In recent years, there has been a growing awareness that some of these "metabolites" of SP also have biological effects, and that these effects must be considered when attempting to understand the actions of SP in the CNS. There is increasing evidence that SP(1–7), and closely related N-terminal fragments, may mediate some of SP's effects in the CNS. In mice, intraventricular (ICV) injections of SP significantly elevated specific components of motor behavior, specifically grooming, scratching, hindlimb rearing, and locomotion (8). SP also appeared to increase dopamine (DA) release in the substantia nigra (SN), since the amount of the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) present, relative to DA, was significantly elevated (7). Similar ICV injections of SP(1–7) likewise elevated rearing and locomotion, but not grooming and scratching. SP(1–7) also elevated the DOPAC-to-DA ratio in the SN (7). In contrast, ICV injections of C-terminal fragments of SP elevate grooming and scratching, but depress rearing and sniffing (8).

The effects of SP in mice, described above, are basically the same as seen in rats following SP microinjections into the SN. Several studies have shown that intra-nigral injection of SP enhances the same components of motor behavior, specifically hindlimb rearing, sniffing, locomotion and, in some cases, grooming (4,11). Such injections are also reported to enhance activity in the nigro-striatal dopaminergic system, resulting in enhanced levels of DA metabolites in the striatum (27). These similarities in behavioral effects, coupled with the similar elevations of DOPAC/DA ratios in the SN, suggest that the SN is also the site of action for SP and SP(1–7) given ICV. The following experiments were performed in order to determine whether SP(1–7) alters motor behavior and DA metabolism via an action on the SN.

METHOD

Animals

Male Sprague-Dawley rats (250–350 g; Charles River) were used in all experiments. They were housed 2–3 per cage, with ad lib access to food and water, under controlled light-dark cycle.

Chemicals

The peptide SP(1–7) was synthesized by the solid phase method (26) and purified by countercurrent distribution. Purity was determined by paper electrophoresis and thin layer chromatography, and composition was determined by amino acid analysis after hydrolysis. Doses were calculated using the found molecular weight for each batch.

In Vivo Experiments

Stainless-steel guide cannulae (23 gauge) were implanted bilaterally with their tips positioned above the SN (3 mm posterior

[1]Requests for reprints should be addressed to Michael E. Hall, Department of Biochemistry, Box B-126, University of Colorado Health Sciences Center, 4200 E. Ninth Ave., Denver, CO 80262.

to bregma; ±2.5 mm from the midline; 7.5 mm below the dura; incisor bar 5.0 mm above the inter-aural line) (17) under pentobarbital anesthesia. One week later, rats were infused bilaterally (2 μl/side; at 1 μl/min plus 30 s for diffusion) with SP(1–7) (0.2 or 2 nmol/side) or vehicle (phosphate-buffered saline; PBS) using stainless-steel needles (30 gauge) inserted 1 mm beyond the tip of the guide cannulae. Infusion was performed in the home cage of the conscious, unrestrained rat and was followed immediately by transfer to the open-field test box. The box (70 × 50 × 50 cm) was internally illuminated (20-watt bulb). An observer, viewing through a one-way window, recorded the frequency and cumulative duration of specific behaviors (grooming, scratching, hindlimb rearing and sniffing) by depressing specific keys on a hand-held keyboard. Each keypress activated a counter and a cumulative timer, which remained activated as long as the key remained depressed. Locomotion was measured in terms of the number of 18 × 18 cm square floor areas traversed. Behavior was recorded continuously for 5 min immediately after injection. All testing was done "blind."

Dye injection experiments were used to determine the extent of peptide diffusion. In most cases, the injectate remained within the SN, where it spread to both the pars compacta and pars reticulata.

Brain Amine Analyses

At the end of observation, all rats were killed by decapitation. Brains were quickly removed on ice and sliced into 500 μm coronal sections at −20°C with a cryotome. A portion of the SN was removed from brain sections using a 16-gauge circular punch tool. Dopamine and DOPAC were separated from brain homogenates using reverse-phase HPLC and quantified by electrochemical detection, as described in detail elsewhere (18). Group differences were analysed by ANOVA and post hoc comparisons made using Duncan's multiple range test (2).

In Vitro Release Experiments

Otherwise untreated rats were decapitated and their brains quickly removed. The SN were removed by dissection from coronal sections and incubated in 1 ml of modified Krebs-bicarbonate (MKB) buffer (bovine serum albumin, 0.06%; ascorbic acid, 170 μM; EDTA, 27 μM; composition in mM: NaCl, 134; KCl 5.0; $CaCl_2$, 2.0; KH_2PO_4, 1.0; $NaHCO_3$, 16; glucose, 10.2; pH 7.4) containing 10 μCi of ^{3}H-DA (New England Nuclear) at 37°C for 15 min. The tissue was oxygenated by continuous bubbling with 98% O_2/2% CO_2. After rinsing with MKB, the tissue was transferred to a nylon mesh screen in a perfusion vessel and superfused continuously (1 ml/min) with oxygenated MKB at 37°. After a 30-min wash-out period, superfusate exiting the vessel was collected at 1-min intervals for 10 min. During the 5th and 6th min, the tissue was superfused with MKB containing SP(1–7) at 10^{-6} M. The ^{3}H-DA in 0.5 ml aliquots of each sample was determined by liquid scintillation counting. The tissue was then solubilized and the ^{3}H-DA remaining was determined by scintillation counting. Tritium release was expressed as a percent of the total tissue content at that time.

RESULTS

Intra-nigral infusion of SP(1–7) led to a significant increase in both the number of times hindlimb rearing occurred, $F(2,56) = 4.0$, $p<0.025$, and in the total duration of rearing behavior, $F(2,56) = 6.66$, $p<0.005$, within the first 5 min after injection (Fig. 1). SP(1–7) also significantly decreased the number, $F(2,58) = 4.23$, $p<0.02$, but increased the duration, $F(2,58) = 8.16$, $p<0.001$, of sniffing events. Square-crossing (locomotor) behavior was significantly, $F(2,57) = 3.78$, $p<0.03$, increased by both doses. SP(1–7) had no statistically significant effects on grooming (Fig. 1) or scratching behavior (data not shown).

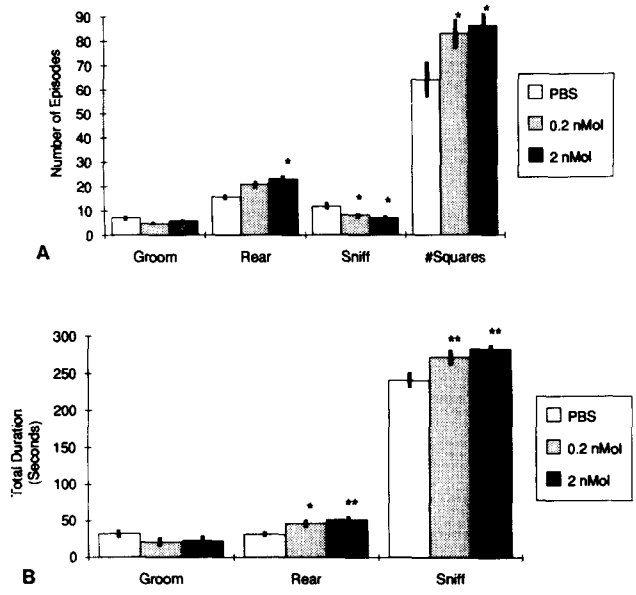

FIG. 1. The effects (mean ± S.E.M.) of bilateral intra-nigral injections of SP(1–7) on (A) the number of episodes, and (B) the total duration of selected components of spontaneous motor behavior. Behaviors were recorded as described in the text during the first 5 min postinjection. *$p<0.05$ and **$p<0.01$, compared to vehicle (PBS) injections.

Infusion of SP(1–7) also produced a significant, $F(2,29) = 4.73$, $p<0.02$, elevation of the DOPAC/DA ratio of the SN from brains removed 5 min after infusion (Fig. 2). SP(1–7) had no statistically significant effects on DA or DOPAC levels.

Superfusion of SN tissue with SP(1–7) in vitro resulted in a significant increase in the release of ^{3}H-DA (Table 1). Tritium efflux increased from about 0.4%/min before exposure, to about 0.6%/min in the presence of 10^{-6} M SP(1–7). No corresponding increase in release was seen in control tissue continuously

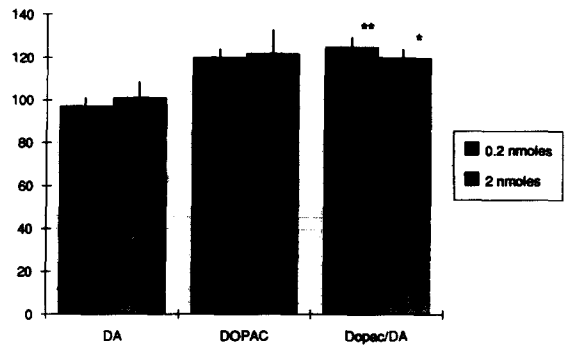

FIG. 2. The effects (mean ± S.E.M.) of intra-nigral injections of 2 (light bars) of 0.2 (dark bars) nanomoles (nmoles) of SP(1–7) on the levels of DA, DOPAC and the DOPAC/DA ratio in the substantia nigra measured 5 min after injection. Levels of all amines are expressed as percent of control levels. *Indicates $p<0.05$ and **indicates $p<0.01$, compared to the PBS-treated control group.

TABLE 1

THE EFFECTS (MEAN ± S.E.M.) OF SP(1–7) (10^{-6} M) ON THE SPONTANEOUS RELEASE OF ^{3}H-DA FROM SN SLICES IN VITRO, EXPRESSED AS THE PERCENT OF TOTAL TISSUE ^{3}H-DA CONTENT AT THAT TIME

Fraction:	1	2	3	4	(5)	(6)	7	8	9	10
Control	0.43 ± 0.01	0.42 ± 0.01	0.42 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.38 ± 0.02	0.37 ± 0.02	0.38 ± 0.02	0.38 ± 0.02	0.38 ± 0.02
SP(1–7)	0.44 ± 0.01	0.42 ± 0.02	0.43 ± 0.02	0.43 ± 0.01	0.60 ± 0.03*	0.56 ± 0.03*	0.44 ± 0.02	0.43 ± 0.02	0.42 ± 0.02	0.41 ± 0.02

SN slices were superfused with a separate MKB solution containing 10^{-6} M SP(1–7) during minutes 5 and 6 of the collection period. Control slices were exposed to a MKB solution not containing peptide. (*$p<0.01$, compared to the corresponding control fractions.)

superfused with MKB not containing SP(1–7).

DISCUSSION

These results show that the N-terminal, nontachykinin fragment SP(1–7) can, like SP itself, significantly enhance rearing, sniffing and locomotion in rats after infusion into the SN. Grooming, which may (4) or may not (12) increase following intra-nigral SP, was certainly not increased by SP(1–7). These effects are largely consistent with the effects of intraventricular injection of SP and SP(1–7) in mice, where both peptides increase rearing and locomotion, but only SP also increases grooming (8). Grooming, on the one hand, and rearing and locomotion, on the other, seem to form two distinct sets of behaviors. Starr and Starr (25) suggested that, in mice, DA agonists may enhance grooming via D1 receptors, while increasing rearing and locomotion via D2 receptors.

SP(1–7) significantly elevated nigral DOPAC/DA ratios in vivo, suggesting that SP(1–7) increased nigral DA release. This interpretation was supported by the direct demonstration that exposure to SP(1–7) significantly increased ^{3}H-DA release from SN tissue in vitro. Other investigators have also reported that intra-nigral injections of SP(1–7) can alter both motor behavior (9) and striatal DA release (9,23), as measured by in vivo microdialysis in anesthetized rats.

In spite of the volume of evidence of significant effects of intra-nigral SP, receptor binding studies have consistently failed to demonstrate SP (NK-1) receptors in the rat SN (3, 14, 21, 22). While there are several plausible explanations for this, one possibility is that the effects of SP are mediated by N- and C-terminal fragments, acting via binding sites that do not recognize intact SP. SP(1–7) reproduced all of the effects seen with SP, except for enhanced grooming and scratching, which other studies have demonstrated are mediated by C-terminal SP fragments (5,19). Endopeptidase 24.11, an enzyme capable of generating SP(1–7), is highly localized in the striato-nigral pathway (16,20), and the production of SP(1–7) from SP within the SN has been demonstrated (9). The recent demonstration of specific binding sites for ^{3}H-SP(1–7) in brain and spinal cord (10) also supports this interpretation.

In conclusion, the present study shows that the N-terminal SP fragment SP(1–7) can alter motor behavior and DA release via an action on the SN. These findings further support the view that SP(1–7), produced in vivo from endogenous SP, mediates a subset of the actions attributed to SP itself, and that the processing of SP into N- and C-terminal fragments is an integral step in the biological actions of SP.

ACKNOWLEDGEMENTS

This work was supported in part by NIH grant NS18531. The authors thank Francis Miley and Robert Binard for their expert technical assistance.

REFERENCES

1. Brownstein, M. J.; Mroz, E.; Kizer, S.; Palkovitz, M.; Leeman, S. Regional distribution of substance P in the brain of the rat. Brain Res. 116:299–305; 1976.
2. Bruning, J. L.; Kintz, B. Computational handbook of statistics. 2nd ed. Glenview, IL: Scott, Foresman & Co.; 1977.
3. Danks, J. A.; Rothman, R.; Cascieri, M. A.; Chicchi, C.; Liang, T.; Herkenham, M. A comparative autoradiographic study of the distributions of substance P and eledoisin binding sites in rat brain. Brain Res. 385:273–281; 1986.
4. Diamond, B. I.; Comaty, J. E.; Sudakoff, G. S.; Havdala, H.; Walter, R.; Borison, R. L. Role of substance P as a "transducer" for dopamine in model choreas. In: Chase, T., ed. Advances in neurology. vol. 23. New York: Raven Press; 1979:505–515.
5. Elliott, P. J.; Iversen, S. D. Behavioral effects of tachykinins and related peptides. Brain Res. 381:68–76; 1986.
6. Erspamer, V. The tachykinin peptide family. Trends Neurosci. 4:267–269; 1981.
7. Hall, M. E.; DeArmey, P.; Stewart, J. M. Effects of an amino-terminal fragment of substance P on brain dopamine and serotonin metabolism, In: Henry, J. L., ed. Substance P and neurokinins. New York: Springer-Verlag; 1987:344–346.
8. Hall, M. E.; Grantham, P.; Limoli, J.; Stewart, J. M. Effects of substance P and neurokinin A (substance K) on motor behavior: unique effect of substance P attributable to its amino-terminal sequence. Brain Res. 420:82–94; 1987.
9. Herrera-Marschitz, M.; Terenius, L.; Sakurada, T.; Reid, M.; Ungerstedt, U. The substance P(1–7) fragment is a potent modulator of substance P actions in the brain. Brain Res. 521:316–320; 1990.
10. Igwe, O.; Kim, D.; Seybold, V.; Larson, A. Specific binding of substance P aminoterminal heptapeptide to mouse brain and spinal cord membranes. J. Neurosci. 10:3653–3663; 1990.
11. Kelley, A. E.; Iversen, S. D. Behavioral response to bilateral injections of substance P into the substantia nigra of the rat. Brain Res. 158:474–478; 1978.
12. Kelley, A. E.; Cador, M.; Stinus, L. Behavioral analysis of the effect of substance P injected into the ventral mesencephalon on investigatory and spontaneous motor behavior in the rat. Psychopharmacology (Berlin) 85:37–46; 1985.
13. Lynch, D.; Snyder, S. Neuropeptides: multiple molecular forms, metabolic pathways, and receptors. Annu. Rev. Biochem. 55:773–799; 1986.
14. Mantyh, P. W.; Maggio, J.; Hunt, S. P. The autoradiographic distribution of kassinin and substance K binding sites is different from the distribution of substance P binding sites in rat brain. Eur. J. Pharmacol. 102:361–364; 1984.
15. Matsas, R.; Fulcher, I.; Kenny, A.; Turner, A. Substance P and [Leu]enkephalin are hydrolysed by an enzyme in pig caudate synaptic membranes that is identical with the endopeptidase of kidney microvilli. Proc. Natl. Acad. Sci. USA 80:3111–3115; 1983.
16. Oblin, A.; Danse, M.; Zivkovic, B. Degradation of substance P by

membrane peptidases in the rat substantia nigra: effect of selective inhibitors. Neurosci. Lett. 84:91–96; 1988.
17. Pellegrino, L. J.; Pellegrino, A.; Cushman, A. J. A stereotaxic atlas of the rat brain. 2nd ed. New York: Plenum Press; 1979.
18. Pelleymounter, M. A.; Fisher, Q.; Schlesinger, K.; Hall, M.; DeArmey, P.; Stewart, J. M. The effects of substance P and its fragments on passive avoidance retention and brain monoamine activity. Behav. Brain Res. 21:119–127; 1986.
19. Piercey, M.; Dobry, P.; Einspahr, F.; Schroeder, L.; Masiques, N. Use of substance P fragments to differentiate substance P receptors of different tissues. Regul. Pept. 3:337–349; 1982.
20. Pollard, H.; Llorens-Cortes, C.; Couraud, J.; Ronco, P.; Verroust, P.; Schwartz, J. Enkephalinase (EC 3.4.24.11) is highly localized to a striatonigral pathway in rat brain. Neurosci. Lett. 77:267–271; 1987.
21. Quirion, R.; Van Dam, T. Multiple tachykinin receptors in guinea pig brain. High densities of substance K (neurokinin A) binding sites in the substantia nigra. Neuropeptides 6:191–204; 1985.
22. Quirion, R.; Van Dam, T. Multiple tachykinin receptors. In: Jordan, C.; Oehme, P., eds. Substance P: Metabolism and biological actions. London: Taylor & Francis; 1985:45–64.
23. Reid, M.; Herrera-Marschitz, M.; Terenius, L.; Ungerstedt, U. Intranigral substance P modulation of striatal dopamine: interaction with N-terminal and C-terminal substance P fragments. Brain Res. 526:228–234; 1990.
24. Sakurada, T.; Greves, P.; Stewart, J.; Terenius, L. Measurement of substance P metabolites in rat CNS. J. Neurochem. 44:718–722; 1985.
25. Starr, B. S.; Starr, M. S. Differential effects of dopamine D1 and D2 agonists and antagonists on velocity of movement, rearing and grooming in the mouse. Neuropharmacology 25:455–463; 1986.
26. Stewart, J. M.; Young, J. D. Solid phase peptide synthesis. 2nd ed. Rockford, IL: Pierce Chemical Co.; 1984.
27. Waldmeier, P. C.; Kam, R.; Stocklin, K. Increased dopamine metabolism in rat striatum after infusions of substance P into the substantia nigra. Brain Res. 159:223–227; 1978.