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Motor Activity Induced by Substance P in Rats

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RONDEAU, D. B., F. B. JOLICOEUR, F. BELANGER AND A. BARBEAU. *Motor activity induced by Substance P in rats.* PHARMAC. BIOCHEM. BEHAV. 9(6) 769–775, 1978.—The effects of intraventricular injections of various doses (20.00, 5.00, 1.25, 0.30 and 0.07 μg) of synthetic Substance P on motor activity in rats were investigated. Activity scores as determined by photocell counts recorded for a 15 min test period were significantly increased in animals injected with 0.30 and 1.25 μg/rat Substance P. The other doses examined did not affect activity. Doses in the range of 40–80 μg produced immobility, rigidity and barrel rotations. Hypersalivation as indicated by a wet fur following an episode of grooming was observed in several animals. Administration of Substance P, in a dose of 0.60 μg, in combination with or 30 min after injections of 2 mg/kg d-amphetamine and 1 mg/kg apomorphine did not potentiate or reduce the increased activity and stereotypy induced by these two drugs.

Substance P Brain peptides Motor activity Stereotypy Hypersalivation Amphetamine Apomorphine

THE influences of brain peptides on physiological functions and on behavior challenge classically held views concerning the relationship between the endocrine and central nervous systems and the functioning of the brain, more specifically regarding chemical transmission between nerve cells [2, 24, 32, 43]. The evidence that a number of peptides present in the brain have direct actions on the central nervous system and play physiological roles in neuronal function has been reviewed recently [1, 2, 41, 43]. Neuropeptides, such as TRH (thyrotropin releasing hormone), α- and β-MSH (melanocyte stimulating hormone), vasopressin, somatostatin, β-endorphin and a few others, have behavioral effects and are candidates for neurological functions. The recent characterization of Substance P as an undecapeptide having the sequence H-Arg-Pro-Lys-Pro-Gln-Phe-Phe-Gly-Leu-Met-NH_2 [6] and the realization of its definite involvement in some biological activities have generated considerable efforts to determine its role in the nervous system [41].

Neural and nonneural activities of Substance P (SP) and its possible roles in the nervous system have been examined comprehensively [27]. Electrophysiological and neurochemical evidence is accumulating that SP acts as a transmitter or modulator of some but not all primary sensory neurons. This hypothesis is based on the localization of the peptide in small sensory neurons in the spinal cord, on the release of immunoreactive SP-like material from slices of the spinal cord as well as on the motoneurons depolarizing properties of the peptide [27]. Peripheral and intracerebral injections of SP produced analgesia in mice; the analgesic effect was prevented by naloxone, a presumed specific antagonist of morphine [14,37]. However, hyperalgesia has been reported following administration of higher doses of SP, alone [28] or in combination with naloxone or baclofen [14], and it has been suggested that the peptide may be a natural antagonist of morphine in the central nervous system [37]. SP also abolished the so-called abstinence syndrome usually provoked by nalorphine injection in morphinized mice [36]. These findings suggested a possible relationship between SP and the recently discovered brain opiate peptides, enkephalins and endorphins [14].

The distribution of SP in the brain is widespread and uneven. SP is found in high concentrations in the hypothalamus and preoptic area [3], two regions of the brain involved in neuroendocrine and neurovegetative functions. Moreover, release of immunoreactive SP-like material from rat hypothalamus slices upon application of a depolarizing stimulus has been demonstrated [20]. Intracerebral administration of SP and eledoisin, a peptide which shares a similar amino acids sequence at the C-terminal, elicited drinking in the pigeon; this behavioral response also followed injections of the peptides into the lateral hypothalamus and preoptic area [12]. However, these two peptides did not cause drinking in rats and were found to depress angiotensin and carbachol induced drinking in this species [13]. The difference in response to SP and eledoisin between rat and pigeon as well as the significance of these observations in relation to

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body fluid regulation are unclear at the present time [12,13]. The influence of SP on the release of pancreatic and anterior pituitary hormones has been reviewed recently [27].

High levels of SP have been detected within the mesencephalon in the interpeduncular nucleus and in the pars reticulata of the substantia nigra [3]. Also, it has been established that the habenulointerpeduncular and striatonigral pathways include SP-containing cells [26]. A series of experiments using selective knife cuts and kainic acid lesions of brain structures has indicated that 90% of the SP in the pars reticulata of the substantia nigra comes from the anterior striatum via the striatonigral pathway [4,19], probably in neurons other than those containing GABA [15].

The possible functional relationships between SP and the monoamines also present in the hypothalamus and substantia nigra in high concentrations have been investigated. It has been shown that SP stimulated both synthesis and utilization of dopamine, noradrenaline and serotonin in whole rat brain; the increasing effects of intraventricular injections of the peptide on dopa and 5-hydroxytryptophan formation were observed at a different dose range [5]. Dopamine turnover was accelerated and serotonin turnover was retarded in mice striatum following peripheral injection of a low dose of SP [33]. Moreover, administration of the undecapeptide into the substantia nigra resulted in the in vivo release of dopamine in the caudate nucleus [7]. This finding was indicative of an excitatory action of SP-containing neurons on dopaminergic cells in the substantia nigra and was in agreement with electrophysiological data which showed that SP produced a slow and small excitation of the neurons in this region [10]. Indirect evidence that SP may exert an excitatory action on nigrostriatal dopaminergic neurons was provided by the observation that intranigral application of SP in rats caused contralateral rotations [21,29], a behavior primarily but not solely attributed to an asymmetric activation of the dopaminergic nigrostriatal pathway [16,39]. Circling was observed also following injection of a six amino acids eledoisin fragment into the substantia nigra [29]. Moreover, administration of SP produced tight head to tail ipsilateral rotations in animals with unilateral 6-hydroxydopamine lesions of the nigrostriatal tract at the level of the lateral hypothalamus pretreated with either amphetamine or apomorphine [8]. This finding suggested that SP, as well as TRH which produced similar effects, may regulate circling behavior by indirectly releasing dopamine. Those two peptides produced barrel rotations in non-lesioned animals without pretreatment [8]. Administration of high doses of SP into the right ventricle of naive rats induced within a few minutes horizontal rotations to the left, and then, rotations along the length axis of the body which were followed by a period of general excitation; the rotations were not observed at doses around 10 μg/rat [5]. Much lower doses of the peptide administered by this route depressed operant responding in rabbits [18,25]. Doses ranging from 0.1 to 3.0 μg/rabbit caused restlessness and increased locomotion [25]. Recently, intraventricular injections of the C-terminal hexapeptide of SP, SP_{6-11}, in a dose of 500 pmol, have been reported to produce an increase in locomotor activity in rats [22]. The purpose of the present experiments was first to establish the dose related effects of intraventricular administration of synthetic SP on motor activity in rats (Experiment 1) and then to examine the possible interaction of the peptide with amphetamine and apomorphine (Experiment 2). Results demonstrated that synthetic SP, in doses of 0.30 and 1.25 μg/rat, produced a significant increase in motor activity. Synthetic SP did not potentiate or inhibit the increased activity and stereotypy induced by amphetamine and apomorphine.

EXPERIMENT 1

METHOD

Animals

Male Sprague-Dawley rats obtained from Canadian Breeding Farm (St-Constant, P. Québec) were used. They were housed in a colony under constant conditions of temperature, humidity and a 12 hr light/dark cycle. Food (Purina Rat Chow) and water were available ad lib. Rats, 275–300 g, were anaesthetized with sodium pentobarbital (60 mg/kg, IP) and chronically implanted with a cannula into the left ventricle according to the published procedure from this laboratory [11]. They were then placed in individual cages. Behavioral testing and drug treatment took place at least 24 hr after surgery.

Procedure

Motor activity was measured using a circular (70 cm in dia.) activity meter (Lehigh Valley Electronics) comprising six photoelectric cells. Activity scores were recorded for periods of 5 min. Animals were observed during these periods and the number of rearings and the time in seconds spent grooming and/or scratching were recorded. Rats were first placed in the apparatus for a 5 min adaptation period and then returned to their individual cages. After 10–20 min, animals received an intraventricular injection of synthetic SP and were placed immediately in the activity meter for 15 min. Behavioral measures were obtained at 5 min intervals. Six groups of 8 rats were tested.

Synthetic SP (Peninsula Laboratories, San Carlos, California) was dissolved in a 0.01 N acetic acid solution previously buffered with NaOH to pH=6.2. One group of animals was injected with the vehicle only and served as a control group. The other groups corresponded to the following doses of SP: 20.00, 5.00, 1.25, 0.30 and 0.07 μg. The injection volume was 20 μl; it was administered over a 2 min period via a 50 μl Hamilton syringe.

RESULTS

Three separate one-way analyses of variance [42] on data collected during the 5 min adaptation period revealed no significant difference between groups for activity scores, number of rearings, and time spent grooming. Therefore, groups were equivalent in terms of the behavioral measures taken prior to intraventricular administration of SP.

The results of the experiment are based on the total activity scores, total number of rearings and total time in seconds spent grooming during the 15 min test session in the activity meter immediately after injection of the peptide. Mean activity scores were significantly different as revealed by a one-way ANOVA, $F(5,42)=2.81$, $p<0.05$. A post hoc Dunnett test [42] indicated that activity scores for the two groups which received, respectively, the 0.30 and 1.25 μg doses of SP were significantly greater than that of the control group. This is illustrated in Fig. 1 in which mean total activity scores during the 15 min test session are plotted for each dose of SP. Although elevated, activity scores for the groups which received the higher doses of the peptide did not differ significantly from that of the group injected with the vehicle only.

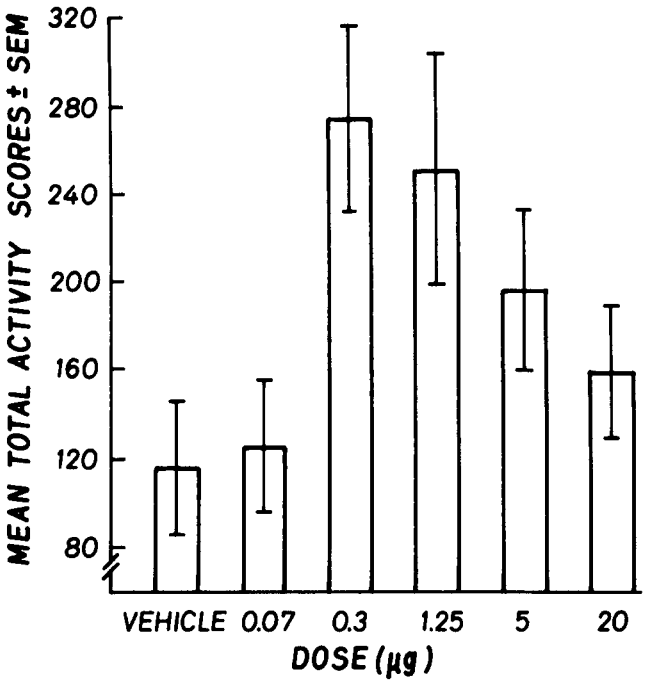

FIG. 1. Mean total activity scores during the 15 min test session following intraventricular administration of Substance P. Eight rats per group.

Mean activity scores for 5 min bins of the test session are presented in Fig. 2. An examination of the data indicated that activity scores of the groups which received injections of 0.30 and 1.25 μg SP were increased during all three 5 min intervals of the session. Differences relative to the control group were greater in the first 5 min of the test session for the 0.30 μg group and in the second 5 min period for the 1.25 μg group. Also, it should be noted that the mean activity score of animals injected with 20.00 μg SP was lower than that of the control group for the first 5 min of the test session. Most rats in this group were completely immobile for 3–4 min after administration of the peptide.

A one-way ANOVA revealed that mean total number of rearings was significant, $F(5,42)=2.85$, $p<0.05$. A post hoc Dunnett test indicated a significant difference between the 0.30 μg and the control groups. Mean total number of rearings during the 15 min test session is presented in Fig. 3 in which it can be seen that the group injected with 0.30 μg SP displayed this behavior much more frequently. The distribution of the number of rearings in 5 min bins parallelled closely that of activity scores for each dose of SP. Another ANOVA indicated no significant difference between groups in total time spent grooming. Mean time in seconds spent grooming during the test session is presented in Table 1 in which it can be noted that, although SP treated animals appeared to spend more time grooming and/or scratching than the control group, the very high variability and the fact that the four higher doses of SP produced similar effects precluded a significant observation. It was noted on several occasions that the fur of some animals was wet. This wet fur was used as a crude indication of hypersalivation. The number of rats in each group which displayed hypersalivation is given in Table 1. Hypersalivation possibly occurred in other animals without being detected visually.

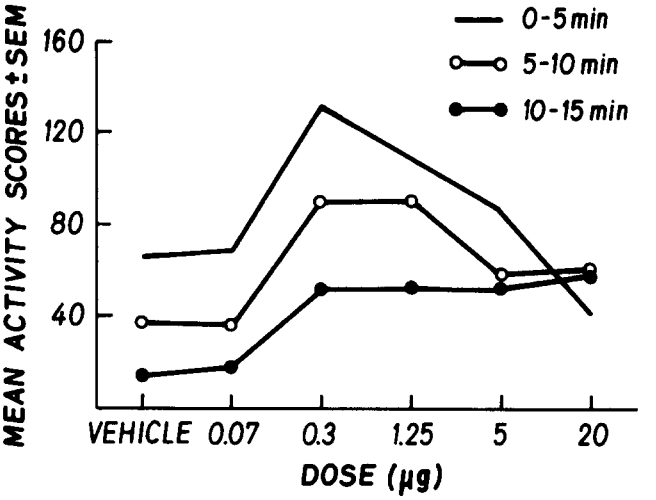

FIG. 2. Mean activity scores for each 5 min intervals of the test sessions.

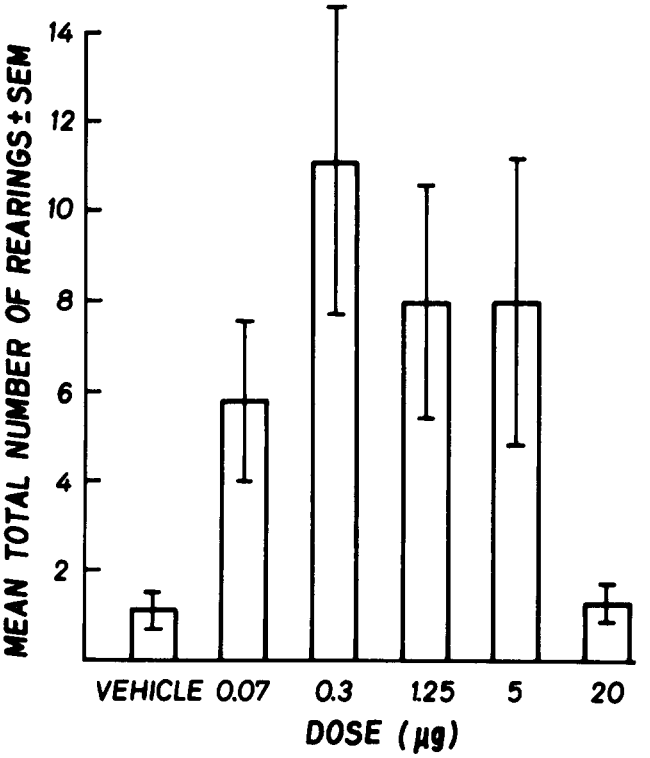

FIG. 3. Mean total number of rearings during the 15 min test session following intraventricular administration of Substance P. Eight rats per group.

EXPERIMENT 2

METHOD

Animals

As in Experiment 1, male Sprague-Dawley rats, 275–300 g, implanted with a cannula into the left ventricle were used.

TABLE 1

Doses of Substance P (μg)	Mean time in sec spent grooming ± S.E.M. during the 15 min session	Number of rats showing hypersalivation (N=8/group)
Vehicle	79.3 ± 31.8	0
0.07	136.1 ± 42.8	1
0.30	206.6 ± 27.9	4
1.25	213.3 ± 46.2	8
5.00	207.3 ± 38.7	7
20.00	208.6 ± 49.0	6

Procedure

Motor activity was measured in four groups of 6 rats placed in the apparatus described above for periods of 5 min at 10 min intervals. During the 10 min intervals animals were removed from the activity meter and placed singly in circular wire-mesh cages. The groups corresponded to the following experimental treaments: IVT injection of 0.60 μg SP and IP injection of 2 mg/kg d-amphetamine (d-amphetamine sulphate, Smith, Kline and French Ltd.); IVT injection of 0.60 μg SP and IP injection of 0.9% NaCl; IVT injection of the vehicle for SP and IP injection of 2 mg/kg d-amphetamine; IVT injection of the vehicle for SP and IP injection of 0.9% NaCl. The injection volume for intraventricular administration was 20 μl. Activity scores were obtained on three occasions before any experimental treatment was applied. Rats received an IVT injection of SP or its vehicle followed by an IP injection of either d-amphetamine or 0.9% NaCl immediately prior to the fourth test session in the activity meter. Motor activity was then recorded at 10 min intervals for seven 5 min test sessions, i.e., sessions 4–10. Upon removal from the apparatus after completion of a test session, animals were placed in individual wire-mesh cages and observed for stereotypy during the following 5 min. Stereotyped behavior was rated according to the scale used by Costall *et al.* [9]: 0—no stereotyped behavior; 1—periodic sniffing and/or repetitive head and limb movements; 2—continuous sniffing and/or repetitive head and limb movements; 3—periodic gnawing, biting or licking; 4—continuous gnawing, biting or licking.

Two more groups of 6 animals were tested according to the procedure described above. One group received an IVT injection of 0.60 μg SP followed by a SC injection of 1 mg/kg apomorphine (apomorphine HCl, MacFarlan-Smith Ltd.) immediately prior to the fourth test session in the activity meter while the other one was injected with the vehicle of SP before apomorphine administration.

RESULTS

Results on motor activity are based on the photocell counts obtained during the first five 5-min sessions in the apparatus which followed the combined administration of SP and d-amphetamine or their vehicles, i.e., test sessions 4–8. A 4×5 repeated measures ANOVA [42] was carried out on the activity scores for each drug treatment and for each of these five sessions. Significant differences were found between activity scores during the test sessions, $F(4,80)=5.21$, $p<0.01$, and more importantly between scores of the various groups of rats, $F(3,20)=13.36$, $p<0.01$. The drug treatment by test sessions interaction was found also to be significant, $F(12,80)=4.20$, $p<0.01$. An analysis of the simple main effects of the factor drug treatment revealed that activity scores were significantly different between the groups for all five sessions, $F(3,65)=3.7$, 9.5, 16.6, 7.9 and 5.6, all $p<0.05$. A post hoc Dunnett test indicated that activity scores in the fourth test session were significantly greater for the group which received IVT 0.60 μg SP and IP 0.9% NaCl than for the control group injected with the SP vehicle and 0.9% NaCl. This significant difference, which was found only during the fourth test session, i.e., immediately after administration of the peptide, is illustrated in Fig. 4 in which mean activity scores of the four groups are presented for several sessions. Therefore, the increase in motor activity produced by SP did not persist and could not be observed beyond the first test session after intraventricular administration of the peptide. Post hoc Tukey (a) tests revealed no significant difference between the SP-d-amphetamine and vehicle-d-amphetamine groups in all five sessions examined. This result demonstrated that SP did not reduce or potentiate the effects of d-amphetamine on motor activity. All the differences found to be significant by the Tukey (a) tests involved comparisons between the two d-amphetamine treated groups and the two groups injected with 0.9% NaCl.

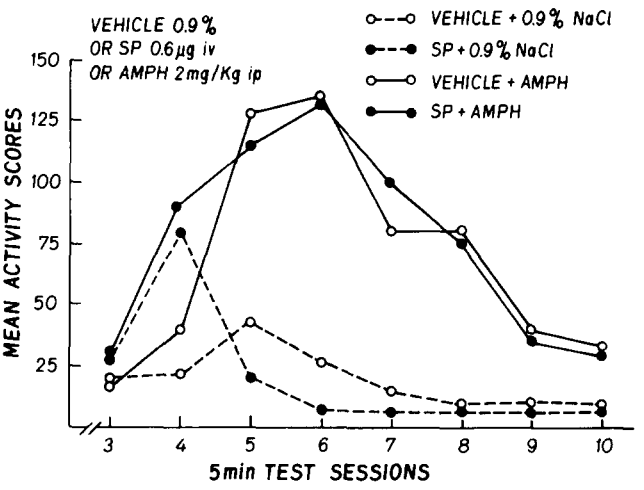

FIG. 4. Mean activity scores of the four groups for several test sessions in the activity meter. Arrow indicates moment of injection of SP, d-amphetamine and/or their vehicles. Six rats per group.

An identical statistical treatment of the data obtained for the animals injected with 1 mg/kg apomorphine demonstrated that SP did not affect the increase in activity produced by this drug. There was no significant difference between the activity scores of the SP-apomorphine and vehicle-apomorphine groups compared by Tukey (a) tests for all five sessions. Mean activity scores of the latter two groups and of the other two groups, the SP-0.9% NaCl and vehicle-0.9% NaCl groups, during the first test session after administration of the peptide, i.e., Session 4, are presented in Fig. 5.

Mean stereotypy scores for groups of animals treated with 2 mg/kg d-amphetamine IP and 1 mg/kg apomorphine

TABLE 2
MEAN STEREOTYPY SCORES

Treatment	N	Time After Injections 0–5	15–20	30–35	45–50	60–65	75–80	90–95
Vehicle + Amphetamine	6	1.0	1.6	1.6	1.0	0.5	0.1	0.0
SP + Amphetamine	6	0.8	1.0	1.5	1.1	0.6	0.1	0.0
Vehicle + Apomorphine	6	2.3	2.6	2.3	0.6	0.0	0.0	0.0
SP + Apomorphine	6	2.3	2.3	2.0	0.6	0.0	0.0	0.0

SP: 0.60 μg; Amphetamine: 2 mg/kg; Apomorphine: 1 mg/kg.

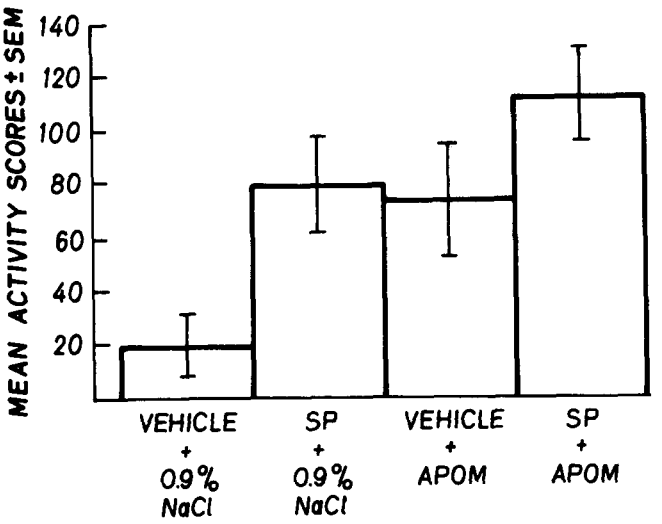

FIG. 5. Mean activity scores during the fourth session. Substance P: 0.60 μg IVT. Apomorphine: 1 mg/kg. Six rats per group.

SC are presented in Table 2. Results demonstrated that intraventricular administration of SP, in a dose of 0.60 μg, did not modify latency, intensity and duration of the stereotyped behavior induced by the two drugs.

Data obtained on 24 additional rats tested under similar conditions with the exception that IVT injections of 0.60 μg SP or its vehicle were given 30 min after administration of d-amphetamine or apomorphine confirmed that SP does not significantly affect the stereotypy and the increase in motor activity induced by these two drugs.

DISCUSSION

The results of Experiment 1 demonstrated that infusion of synthetic SP, in doses of 0.30 and 1.25 μg, into the left ventricle caused an increase in motor activity in rats tested for 15 min in an activity meter. Higher activity scores were recorded during all three 15 min bins of the session for these two groups. Observation of the animals indicated that the increased activity consisted of well coordinated locomotion, frequent rearings and, in some cases, vigorous episodes of grooming. In Experiment 2, an intermediary dose of SP, 0.60 μg, also produced an augmentation in activity scores for a 5 min period immediately after administration of the peptide. In the latter case, the effect did not persist during the next test session, 10 min later.

The behavioral changes observed in the present experiments are in agreement with those reported in rabbits following intraventricular injection of similar doses of synthetic SP [25] and in rats after injection by this route of 500 pmol of SP_{6-11}, the C-terminal hexapeptide of SP [22]. However, it should be mentioned that peripheral administration of synthetic SP produced behavioral depressant effects in mice [33, 35, 36]. A dose of 5 μg decreased locomotor activity and counteracted amphetamine-induced hyperactivity in mice [33]. Moreover, it abolished the motor hyperactivity induced by the neurolathyrogen β-β′-iminodipropionitrile (IDPN) in mice, presumably by raising glycine levels in the spinal cord [35]. The depressant effects of SP administered peripherally in mice have been confirmed in this laboratory (unpublished data).

Circling behavior and barrel rotations were not observed at any of the doses examined in the present experiments. However, most animals which received injections of 20.00 μg SP were completely immobile for 3–4 min. Moreover, a rigid posture with extension of the limbs, interrupted by several barrel rotations, as described by others [5, 8], was a characteristic behavioral response to intraventricular infusion of SP in the 40–80 μg dose range (unpublished observations). Such high doses of the peptide have been reported to depress self-stimulation in rats [17].

Copious secretion of saliva in anesthetized rats was the biological activity used to isolate and characterize SP from bovine hypothalamic extracts [23]. It is interesting to note that hypersalivation, for which a wet fur after an episode of grooming was a crude index, was seen in several rats following intraventricular injections of synthetic SP at different doses.

Injection of 0.60 μg SP, a dose which significantly increased motor activity, did not potentiate or reduce stereotyped behavior and increased activity induced by 2 mg/kg d-amphetamine and 1 mg/kg apomorphine. The negative results were obtained both when the peptide was administered in combination with and 30 min after peripheral injections of the drugs. This finding contrasts with the observations that intramuscular administration of synthetic SP abolished tremors in mice treated with 5 mg/kg amphetamine [34] and that IP injections of a low dose of the peptide counteracted amphetamine-induced hyperactivity in this species [33]. SP concentration in the rat striatum, but not in the substantia nigra, was lowered significantly after injection of 10 mg/kg d-amphetamine; this reduction in striatal levels of the peptide did not occur before 2 hr following treatment with d-amphetamine [30]. Therefore, it can be assumed that there were no decreases in SP concentration in these two

regions of the brain when rats were tested in Experiment 2. The difference in response to SP between mice and rats pretreated with amphetamine is unclear at the present time. It should be mentioned that SP was found to show little activity in the Dopa Potentiation Test in mice [31]. Since single doses of SP, d-amphetamine and apomorphine were examined, it cannot be ruled out that the latter two drugs may interact with SP at some other doses. The results of the present experiments support the hypothesis that SP may be involved in the control of motor functions.

REFERENCES

1. Barbeau, A., M. Gonce and A. J. Kastin. Neurologically active peptides. *Pharmac. Biochem. Behav.* **5:** Suppl. 1, 159–163, 1976.
2. Barker, J. L. Physiological roles of peptides in the nervous system. In: *Peptides in Neurobiology,* edited by H. Gainer. New York: Plenum Press, 1977, pp. 295–343.
3. Brownstein, M. J., E. A. Mroz, J. S. Kiser, M. Palkovits and S. E. Leeman. Regional distribution of substance P in the brain of the rat. *Brain Res.* **116:** 299–305, 1976.
4. Brownstein, M. J., E. A. Mroz, M. L. Tappaz and S. E. Leeman. On the origin of substance P and glutamic acid decarboxylase (GAD) in the substantia nigra. *Brain Res.* **135:** 315–323, 1977.
5. Carlsson, A., T. Magnusson, G. H. Fisher, D. Chang and K. Folkers. Effect of synthetic substance P on central monoaminergic mechanisms. In: *Substance P,* edited by U. S. von Euler and B. Pernow. New York: Raven Press, 1977, pp. 201–205.
6. Chang, M. M. and S. E. Leeman. Isolation of a sialogogic peptide from bovine hypothalamus and its characterization as substance P. *J. Biol. Chem.* **245:** 4784–4790, 1970.
7. Cheramy, A., A. Nieoullon, R. Michelot and J. Glowinski. 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 cat. *Neurosci. Lett.* **4:** 105–109, 1977.
8. Cohn, M., M. L. Cohn, J. A. Feldman and D. Taube. Tyrotropin-releasing hormone (TRH): an endogenous amphetamine-like compound. *Fedn Proc.* **37:** 665, 1978.
9. Costall, B., C. D. Marsden, R. J. Naylor and C. J. Pycock. Stereotyped behaviour patterns and hyperactivity induced by amphetamine and apomorphine after discrete 6-hydroxydopamine lesions of extrapyramidal and mesolimbic nuclei. *Brain Res.* **123:** 89–111, 1977.
10. Davies, J. and A. Dray. Substance P in the substantia nigra. *Brain Res.* **107:** 623–627, 1976.
11. Donaldson, J., T. St-Pierre, J. Minnich and A. Barbeau. Seizures in rats associated with different cation inhibition of Na^+-K^+-ATPase. *Can. J. Biochem.* **49:** 1217–1221, 1971.
12. Evered, M. D., J. T. Fitzsimons and G. De Caro. Drinking behaviour induced by intracranial injections of eledoisin and substance P in the pigeon. *Nature* **268:** 332–333, 1977.
13. Fitzsimons, J. T. and M. D. Evered. Eledoisin, substance P and related peptides: intracranial dipsogens in the pigeon and antidipsogens in the rat. *Brain Res.* **150:** 533–542, 1978.
14. Frederickson, R. C. A., V. Burgis, C. E. Harrell and J. D. Edwards. Dual actions of substance P on nociception: Possible role of endogenous opioids. *Science* **199:** 1359–1362, 1978.
15. Gale, K., J. S. Hong and A. Guidotti. Presence of substance P and GABA in separate striatonigral neurons. *Brain Res.* **136:** 371–375, 1977.
16. Glick, S. D., T. P. Jerussi and L. N. Fleischer. Turning in circles: the neuropharmacology of rotation. *Life Sci.* **18:** 889–896, 1976.
17. Goldstein, J. M. and J. B. Malick. Effects of substance P on medial forebrain bundle self-stimulation in rats following intracerebral administration. *Pharmac. Biochem. Behav.* **7:** 475–478, 1977.
18. Graeff, F. G. and E. A. L. Arisawa. Effect of intracerebroventricular bradykinin, angiotensin II and substance P on multiple fixed-interval fixed-ratio responding in rabbits. *Psychopharmacology* **57:** 89–95, 1978.
19. Hong, J. S., H.-Y. T. Yang, G. Racagni and E. Costa. Projections of substance P containing neurons from neostriatum to substantia nigra. *Brain Res.* **122:** 541–544, 1977.
20. Iversen, L. L., T. Jessell and I. Kanazawa. Release and metabolism of substance P in rat hypothalamus. *Nature* **264:** 81–83, 1976.
21. James, T. A. and M. S. Starr. Behavioural and biochemical effects of substance P injected into the substantia nigra of the rat. *J. Pharm. Pharmac.* **29:** 181–182, 1977.
22. Kubicki, J. Some behavioral effects of SP_{6-11} hexapeptide C-terminal of substance P injected into the cerebral ventricles in rats. *Acta Physiol. pol.* **28:** 489–492, 1977.
23. Leeman, S. E. and R. E. Carraway. Discovery of a sialogogic peptide in bovine hypothalamic extracts: its isolation, characterization as substance P, structure and synthesis. In: *Substance P,* edited by U. S. von Euler and B. Pernow. New York: Raven Press, 1977, pp. 5–13.
24. Martin, J. B., L. P. Renault and P. Brazeau. Hypothalamic peptides: New evidence for peptidergic pathways in the C.N.S. *Lancet* **1:** 393–395, 1975.
25. Melo, J. C. and F. G. Graeff. Effect of intracerebroventricular bradykinin and related peptides on rabbit behavior. *J. Pharmac. exp. Ther.* **193:** 1–10, 1975.
26. Mroz, E. A., M. J. Brownstein and S. E. Leeman. Distribution of immunoassayable substance P in the rat brain: evidence for the existence of substance P-containing tracts. In: *Substance P,* edited by U. S. von Euler and B. Pernow. New York: Raven Press, 1977, pp. 147–154.
27. Mroz, E. A. and S. E. Leeman. Substance P. *Vitam. Horm.* **35:** 209–281, 1977.
28. Oehme, P., J. Bergmann, M. Bienert, H. Hilse, L. Piesche, P. Minh Thu and E. Scheer. Biological action of substance P—Its differentiation by affinity and intrinsic efficacy. In: *Substance P,* edited by U. S. von Euler and B. Pernow. New York: Raven Press, 1977, pp. 327–335.
29. Olpe, H. R. and W. P. Koella. Rotatory behavior in rats by intranigral application of substance P and an eledoisin fragment. *Brain Res.* **126:** 576–579, 1977.
30. Pettibone, D. J., R. J. Wurtman and S. E. Leeman. D-amphetamine administration reduces substance P concentration in the rat striatum. *Biochem. Pharmac.* **27:** 839–842, 1978.
31. Plotnikoff, N. P. and A. J. Kastin. GH-RIF, LH-RH, TRH, MIF-I, Substance P and Angiotensin—Central nervous system studies In: *Peptides: Chemistry, Structure and Biology,* edited by R. Walter and J. Meinhofer. Ann Arbor: Ann Arbor Science Publ., 1975, pp. 645–649.
32. Sandman, C. A., L. H. Miller and A. J. Kastin. Introduction: Perspectives on the behavioral effects of the neuropeptides. In: *Neuropeptide Influences on the Brain and Behavior. Advances in Biochemical Psychopharmacology, Vol. 17,* edited by L. H. Miller, C. A. Sandman and A. J. Kastin. New York: Raven Press, 1977, pp. 1–9.
33. Starr, M. S., T. A. James and D. Gaytten. Behavioural depressant and antinociceptive properties of substance P in the mouse: Possible implication of brain monoamines. *Eur. J. Pharmac.* **48:** 203–212, 1978.
34. Stern, P. The influence of synthetic "Substance P" on tremor caused by LSD in mice. *J. Pharm. Pharmac.* **25:** 259–260, 1973.
35. Stern, P., S. Ćatović and M. Stern. Mechanism of action of substance P. *Naunyn-Schmiedeberg's Arch. exp Path. Pharmack.* **281:** 233–239, 1974.

36. Stern, P. and J. Hadzović. Pharmacological analysis of central actions of synthetic substance P. *Arch. int. Pharmacodyn. Thér.* **202:** 259–262, 1973.
37. Stern, P., S. Huković and M. Radivojević. The inhibition of the effects of morphine by synthetic substance P. *Experientia* **32:** 1326–1327, 1976.
38. Stewart, J. M., C. J. Getto, K. Neldner, E. B. Reeve, W. A. Krivoy and E. Zimmerman. Substance P and analgesia. *Nature* **262:** 784–785, 1976.
39. Ungerstedt, U. Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiol. scand.* **82:** (Suppl. 367), 67–93, 1971.
40. Vale, W., C. Rivier and M. Brown. Regulatory peptides of the hypothalamus. *Ann. Rev. Physiol.* **39:** 473–527, 1977.
41. Von Euler, U. S. and B. Pernow (editors). *Substance P.* New York: Raven Press, 1977.
42. Winer, B. J. *Statistical Principles in Experimental Design,* second edition. New York: McGraw-Hill, 1971.
43. Zetler, G. The peptidergic neuron: a working hypothesis. *Biochem. Pharmac.* **25:** 1817–1818, 1976.