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Intranigral Tachykinin NK3 Receptor Agonist Elicits Oral Movements in Rats

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LIMINGA, U., P. E. JOHANSSON AND L. GUNNE. *Intranigral tachykinin NK3 receptor agonist elicits oral movements in rats.* PHARMACOL BIOCHEM BEHAV **38**(3) 617–620, 1991.—Synthetic agonists for the tachykinin NK1 and NK3 receptors were bilaterally infused at three dose levels (4.2, 0.17, and 0.007 nmol) into each substantia nigra of freely moving rats and oral behaviors were monitored for 30 min postinfusion. It was found that all doses of senktide, an agonist at the NK3 receptor, induced a significant increase of nonobject-directed chewing, vacuous chewing movements (VCM). The highest dose of senktide produced the greatest effect ($p<0.001$) and precipitated wet shakes for about 15 min after infusion. Septide, selective at the NK1 receptor, was without effect on oral behavior. The present results suggest that NK3 receptor-active peptides might be symptom inducers in oral dyskinesia.

Tachykinin analogues Senktide Septide Intranigral infusion Substantia nigra Oral movements Tardive dyskinesia Rat

ORAL movements can be elicited in rats by bilateral infusion of the GABA antagonists bicuculline or isoniazid into the substantia nigra (6). Since the tachykinin substance P (SP) has been shown to be a naturally occurring GABA antagonist in this area (18, 22, 23), we have performed a series of bilateral intranigral infusions of tachykinin analogues in rats in order to investigate peptidergic mechanisms for oral dyskinesia. Two agents were chosen for the present study: The NK1 receptor agonist septide [($pGlu^6$,Pro^9) SP_{6-11}] (14,20) and the NK3 receptor agonist senktide [succinyl-($Asp^6$$MePhe^8$) SP_{6-11}] (3, 14, 29). We decided to study a wide range of doses, since intranigral SP injections have been reported to produce a biphasic dose-response in experiments where striatal dopamine release was monitored (22). Bilateral intranigral infusions were performed in awake rats and behavioral studies were focused on oral movements, which were studied for 30 min postinfusion.

METHOD

Animals and Surgery

Female Sprague-Dawley rats (250–325 g) were housed two in each cage under standardized conditions (room temperature 20–22°C, 12-h light/dark cycle) with free access to food and water. The rats were anesthetized with ether and implanted bilaterally, using a stereotaxic technique, with permanent 22-gauge stainless steel guide cannulae, which ended 3 mm above the infusion site to avoid tissue damage. Stereotaxic coordinates for the placement of the 28-gauge internal cannulae were: 3.2 mm caudal from bregma, 2.0 mm from midline and 7.9 mm below dura (21) with the incisor bar placed 5 mm above the interaural line. After surgery the rats were allowed at least 2 days' recovery.

Infusions

Bilateral infusions were performed on freely moving rats in a plastic cage 40 × 25 × 15 cm, with mirrors to enhance the visual observations. Internal 28-gauge cannulae, which protruded 3 mm beyond the guide cannulae, were connected by polyethylene tubing to 50 μl syringes driven by infusion pumps (Sage Instruments). Drug solution, in a volume of 0.5 μl, was infused for 5 min simultaneously in both substantia nigra areas. The cannulae were removed 1 minute after the end of infusion. Each rat underwent a maximum of two infusions at 24-hour intervals.

Drugs

The rats received 4.2, 0.17 or 0.007 nmol of senktide or septide (Bachem Feinchemikalien AG, Switzerland) on each side in 0.5 μl solvent during the 5-min infusion. Senktide was dissolved in saline, while the solvent for septide was saline containing 10% dimethylsulfoxide (DMSO). Control animals were infused with either 0.5 μl of saline or 10% DMSO in saline.

The following number of successful experiments were performed: Saline n = 6, 10% DMSO n = 9, senktide 4.2 nmol n = 9, 0.17 nmol n = 10, 0.007 nmol n = 9, septide 4.2 nmol n = 8, 0.17 nmol n = 7, 0.007 nmol n = 7.

The purity and stability of senktide and septide solutions were confirmed by means of continuous flow fast atom bombardment (CF-FAB) mass spectrometry using a Finnigan MAT 90 mass spectrometer.

Behavioral Studies

Oral behavior was monitored 2 min before the start of infusion with the internal cannulae inserted and at 1-min intervals from 3 to 30 min postinfusion. For the statistical analysis a mean was calculated for consecutive 2-min periods. The number of vacuous chewing movements (VCM) and wet shakes (WS) (28) were counted.

TABLE 1

VACUOUS CHEWING MOVEMENTS (VCM) OBSERVED DURING 30 MIN AFTER BILATERAL INTRANIGRAL INFUSION OF SENKTIDE OR SEPTIDE

Substance	Dose (nmol)	Max. Eff. (VCM/min)	Minutes to Max	AUC
Saline	0.5 µl	5.6 ± 1.5	16	104 ± 11
DMSO 10%	0.5 µl	4.5 ± 1.6	14	71 ± 16
Senktide	4.2	16.0 ± 4.7†	4	306 ± 26‡
Senktide	0.17	13.0 ± 4.6	10	195 ± 32*
Senktide	0.007	12.3 ± 3.8	12	211 ± 53*
Septide	4.2	6.4 ± 1.4	24	112 ± 14
Septide	0.17	5.5 ± 1.3	16	73 ± 10
Septide	0.007	7.3 ± 2.1	20	134 ± 19

Means ± SE.

*Differs from saline, $p<0.05$; †differs from saline, $p<0.01$; ‡differs from saline, $p<0.001$.

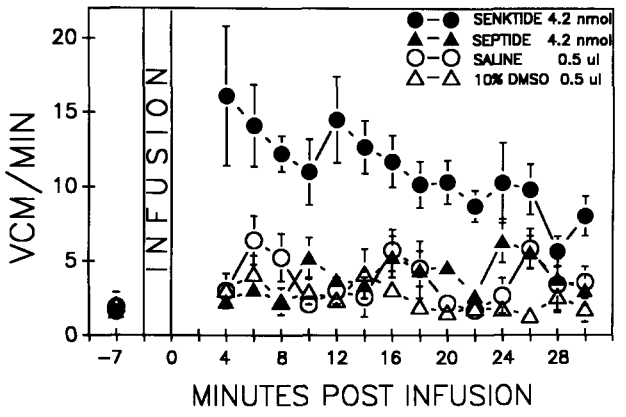

FIG. 1. Vacuous chewing movements (VCM) after bilateral infusion of 4.2 nmol of senktide or septide into each substantia nigra of freely moving rats compared to control treatment, either saline or saline with 10% DMSO, 0.5 µl.

Histological Verification

To facilitate the location of the cannula tips, a small volume (0.1–0.5 µl) of methylene blue was injected right after the decapitation through the cannulae. The whole head of the rat was sliced in a cryo-microtome and the intranigral position was established by observers unaware of the outcome of the behavioral studies. Only animals with both cannula tips in substantia nigra reticulata were included in the study.

Statistics

Data were subjected to two-way analysis of variance with repeated measurements and subsequent Tukey tests (2). The between-subject factor was group, with 8 levels, and the within-subject factor was time, with 14 levels. In addition, areas under the VCM/min—time curve (AUC) were calculated by the trapezoid rule (t2 − t1) × (y1 + y2)/2 (5), and means compared using a one-way analysis of variance. Student's *t*-tests were used for post hoc analysis.

RESULTS

All rats that were tested had a low baseline VCM rate, 1.7 ± 0.2 VCM/min, before the infusions.

Controls

There were no significant differences between the two vehicles. The saline-infused animals had a mean rate of 3.7 ± 1.4 VCM/min and the 10% DMSO-infused animals 2.6 ± 0.9 VCM/min throughout the 30-min observation time.

Senktide

Senktide caused an elevation of the frequency of oral movements, with a peak effect of 16 VCM/min seen 4 min postinfusion (Fig. 1). There was a significant main group effect, $F(7,64) = 8.15$, $p<0.0001$, and subsequent Tukey tests showed that the high dose of senktide (4.2 nmol) differed both from the saline-infused group and all doses of septide ($p<0.01$). All doses of senktide gave a significant increase of the AUC (4.2 nmol: $p<0.001$, 0.17 and 0.007 nmol: $p<0.05$; Table 1).

The highest dose of senktide induced wet shakes between 6–16 min after the end of infusion with a peak effect of 2.9 WS/min at 8 min (Fig. 2). This was not seen at the lower doses of senktide or after septide administration. In rats infused with the highest dose of senktide several bursts of teeth chattering occurred.

Septide

Septide did not induce significant changes in oral behavior at any dose tested.

DISCUSSION

The present findings showed that bilateral intranigral stimulation of the NK1 and NK3 receptor had different effects on oral behavior in freely moving rats. Senktide, the NK3 receptor agonist, elicited an increase in VCM over a wide range of doses. Although the highest dose of senktide (4.2 nmol) had the greatest effect, both 1/25 (0.17 nmol) and 1/625 (0.007 nmol) of this dose gave significant increases of AUC for the VCM/min—time curve. Septide, on the other hand, was without effect on oral behavior in all doses tested.

The present study was designed to elucidate some mechanisms underlying tardive dyskinesia. We have earlier shown that irreversible dyskinesia, produced by chronic neuroleptic treatment in *Cebus apella* monkeys for 2–8 years, is related to regional depressions of brain GABA levels and glutamate decarboxylase (GAD) activities (8,13). Such decreases of GABA occur in substantia nigra, the medial segment of globus pallidus and the subthalamic nucleus. A corresponding reduction of nigral GAD activity was sometimes seen in rats given chronic neuroleptics for 10–18 months (7, 12, 19). However, rats have too short a life span to establish whether a dyskinetic sign is actually irreversible. Further, the oral movements produced by chronic neuroleptics in rats simply consist of an increased rate of VCMs, which are part of a rat's normal behavioral repertoire. Bilateral intranigral infusion of GABA antagonists was shown to produce a temporary rise in VCM, a finding that supports the notion of an impaired function of nigral GABA for the occurrence of oral dyskinetic behavior (6). Together, these findings raise the question whether naturally occurring substances, acting in their effects antagonistic to GABA, may elicit similar signs. In a report where intranigral infusions of SP analogues were examined, it was concluded that SP in the substantia nigra may have effects in the opposite direction compared to nigral GABA (18). Further, in studies using microdialysis techniques, the intranigral application of GABA was shown to have opposite effects on striatal dopamine release compared to the tachykinins SP and neurokinin A (active respectively on NK1

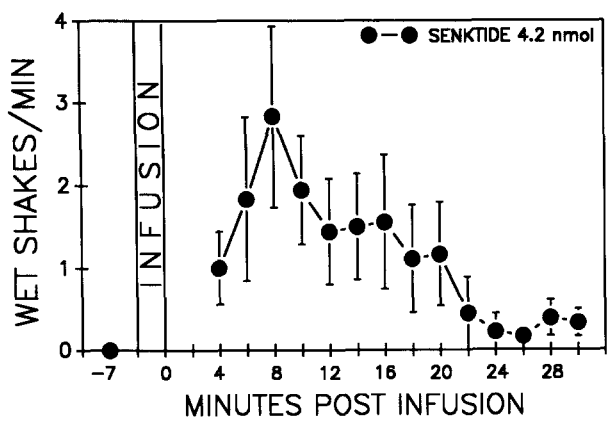

FIG. 2. Wet shakes after bilateral infusion of 4.2 nmol of senktide into each substantia nigra.

and NK2 receptors) (22,23). In the present study senktide induced an effect on oral movements in a wide range of doses (4.2–0.007 nmol). Intranigral application of SP has been reported to affect striatal dopamine as well as rotational behavior over a corresponding wide range of doses (10,23).

Other peptides infused in the nigral area have also been shown to affect oral behavior. In a recent study, we found that bilateral intranigral application of stable Leu- and Met-enkephalin analogues produced a massive oral activity (16). The intense enkephalin-mediated syndrome appeared to be qualitatively different from the unconspicuous VCM rise seen after intranigral senktide application. These two behaviors will be further analysed using a novel technique, which may discriminate between acute dystonia-like and tardive dyskinesia-like mouth movements in rats (25).

Haloperidol given in subchronic experiments on rats was shown to decrease nigral SP content, and the possibility of an enhanced neuroleptic-induced SP release in substantia nigra was discussed (11). Other groups have reported evidence for a decreased nigral biosynthesis and release of tachykinins in rats treated with haloperidol for 10 days (1, 15, 17). However, the relevance of these 1–2-week experiments for the understanding of late complications like tardive dyskinesia is necessarily limited. Our studies of a monkey model for tardive dyskinesia showed that animals which did not develop dyskinetic symptoms, despite years of neuroleptic administration, had a high content of SP in the caudate nucleus and nucleus accumbens (13), while monkeys with tardive neuroleptic-induced dyskinesia had normal SP levels. It was speculated that the increased striatal SP levels could be related to a specific neurochemical response in animals resistant to the dyskinesia-inducing effects of neuroleptics. In these monkeys a downregulation of tachykinin release may have occurred, which protected them from developing dyskinesia. According to the present results, NK3-active peptides might be candidates for such dyskinesia-inducing mechanisms.

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