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BRIEF COMMUNICATION

Intra-Midbrain Raphe Injections of the Neurokinin-3 Agonist Senktide Inhibit Food and Water Intake in the Rat[1]

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PARIS, J. M., H. MITSUSHIO AND S. A. LORENS. *Intra-midbrain raphe injections of the neurokinin-3 agonist senktide inhibit food and water intake in the rat.* PHARMACOL BIOCHEM BEHAV **38**(1) 223–226, 1991.—Microinjection and lesion studies have implicated the midbrain dorsal (DR) and median raphe (MR) nuclei in behavioral arousal. This behavioral state is manifested as locomotor hyperactivity, hyperphagia, hyperdipsia and increases in plasma corticosteroid release. Intra-midbrain raphe injections of the $GABA_A$ agonist muscimol elicit this behavioral activation. We have demonstrated that similar infusions of tachykinins produce locomotor hyperactivity through activation of neurokinin-3 (NK-3) receptors located on serotonin cell bodies. The purpose of the present study was to determine the effects of intra-MR and DR infusions of senktide, an NK-3 agonist, on food and water consumption in nondeprived rats. Male Sprague-Dawley rats were implanted with indwelling intra-MR or intra-DR cannula. Infusions of muscimol (25 ng/0.5 μl) into the MR increased water intake, while MR and DR infusions increased food consumption. In contrast, intra-MR injections of senktide decreased water intake and intra-MR and DR injections decreased food intake. The results suggest that the behavioral states induced by muscimol and neurokinin infusions into the raphe are distinct and that raphe/neurokinin pathways are involved in consummatory mechanisms.

Neurokinins Senktide Neurokinin receptors Median raphe nucleus Dorsal raphe nucleus Food intake Water intake Nondeprived subjects

SEROTONIN (5-hydroxytryptamine; 5-HT) has been implicated in eating (15,33) and 5-HT dysfunction is thought to underlie the pathology of eating disorders (22). Enhancement of 5-HT neurotransmission within the hypothalamus is thought to be inhibitory on food consumption (15). Indeed, peripheral and central injections of the 5-HT_{1A} agonist 8-hydroxy-n-dipropylaminotetralin (8-OHDPAT), which depresses the spontaneous activity of 5-HT cells (30), stimulate feeding in nondeprived rats (8). The 5-HT innervation of the hypothalamus and the entire forebrain originates in the 5-HT perikarya located in the midbrain dorsal (DR) and median (MR) raphe nuclei (9, 16, 17). There is a great deal of evidence implicating the MR and DR in behavioral arousal. Mechanical and chemical lesions of this region produce locomotor hyperactivity (1, 7, 9, 13, 17). Microinjections of the $GABA_A$ agonist muscimol induce dose-dependent increases in activity levels, feeding and drinking, and stimulation of adrenocorticoid secretion (10–12, 14, 29). Interestingly, however, the behavioral effects of muscimol or raphe lesions are not mediated through 5-HT neurons (1, 13, 16, 17, 24, 36).

We have demonstrated that microinjections of neurokinins and neurokinin analogues produce dose-dependent increases in locomotor activity (23–26). This effect is mediated primarily through stimulation of neurokinin-2 and -3 (NK-2 and NK-3) receptors and depends upon intact 5-HT neurons (25–27). Neurokinins have also been implicated in consummatory behaviors in the rat (5, 18–21, 28). Since intra-raphe injections of muscimol and 8-OHDPAT stimulate eating and drinking, the purpose of the present experiment was to determine whether similar injections of the NK-3 agonist senktide induce these behaviors in nondeprived rats.

METHOD

Subjects

Male Sprague-Dawley rats (Sasco-King, Orange, WI), 90–120 days old and 275–325 g, were housed individually in an illumination (12-h light-dark cycle, lights on at 0700 h), temperature-

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and humidity-controlled facility. Food and water were available ad lib. Upon arrival in the laboratory, the animals were allowed at least one week to adjust to their new environment prior to surgery.

Surgery

All cannula implantation procedures have been detailed previously (24). Briefly, the rats were anesthetized with sodium pentobarbital (60 mg/kg, IP), and treated with atropine sulfate (0.4 mg/kg, IM) and sodium ampicillin (50 mg/kg, IM). Using standard stereotaxic techniques, a sterile stainless steel guide cannula (0.46 mm, o.d. and 0.25 mm, i.d.; Plastic One, Roanoke, VA) was lowered at an angle 25° lateral to the mid-sagittal plane, 3.8 mm lateral, 7.8 mm ventral and 7.5 mm caudal from bregma for the MR and 3.2 mm lateral, 6.2 mm ventral for the DR (27). The guide cannula contained a sterile stainless-steel stylet which extended 0.5 mm beyond the tip. The cannula was secured to the skull by means of acrylic cement and stainless-steel screws. All animals were allowed to recover for one week prior to the initiation of behavioral testing.

Behavioral Testing

Measurements of food and water consumption were made in rectangular Plexiglas chambers (45 cm long × 45 cm high × 30 cm wide) with wire mesh floors. A 20-W light bulb located 1.0 m above the chambers provided illumination. One end of the chamber was equipped with a water bottle and a small cup containing crushed rat chow. A petri dish was affixed under the food cup to collect spillage. Activity counts were generated by interruption of a photocell beam located at the base of the chamber and recorded on a digital counter. The rats were handled daily beginning 7–10 days after surgery and were habituated to the testing chamber for 3 days prior to testing. Each animal was gently wrapped in a towel for 30–60 s and placed into the chamber for 30 min. Food and water were available in the chamber. All behavioral testing was conducted between 0900–1400 h, and each rat was always tested in the same chamber. Following the three-day habituation period, drug testing was begun. Vehicle (1.0 μl), senktide (3, 10, 30 pmol) and muscimol (25 ng) were administered in a random order every second day.

The injection procedure consisted of removing the animal from its home cage and gently wrapping it in a towel. The stylet was removed and replaced with an internal cannula connected via polyethylene tubing to a 25 μl Hamilton syringe driven by a microsyringe pump. The drugs and vehicle were administered at a rate of 0.5 μl/30 sec. The volume of all infusions was 1.0 μl and the total time for each infusion was 1.0 min. Upon completion of the injection, the internal cannula was left in situ for an additional 30 s, after which time it was removed and the stylet replaced. During the 30-min testing period, activity counts (photobeam breaks), amount of food eaten (grams; corrected for spillage) and water consumed (ml) were measured.

Histology

Following the completion of the experiment, all rats were sacrificed by an overdose of sodium pentobarbital and transcardially perfused with saline and 10% buffered formalin. The brains were removed and stored in formalin for at least a week. Sections (50 μm) were made through the region of the midbrain raphe, stained with cresyl violet acetate, and evaluated to determine cannula placement. Any animal whose cannula placement was confirmed to be outside of the desired nucleus was eliminated from the study.

Data Analysis

All data were subjected to a repeated-measures analysis of variance (ANOVA) with post hoc comparisons of means made with Duncan's multiple range test (35). Data were considered to be significant when the probability (p) was less than or equal to 0.05.

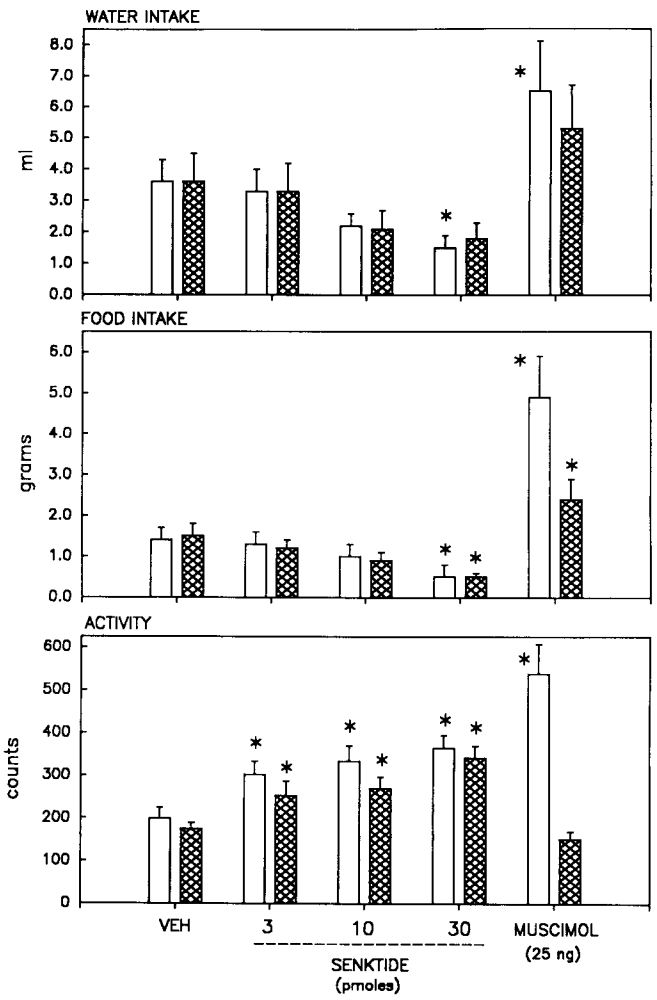

FIG. 1. The effects of intra-median (MR; N = 10) and dorsal (DR; N = 12) raphe infusions of muscimol (25 ng in 0.5 μl vehicle) and senktide (3, 10, 30 pmol in 0.5 μl) on water (top panel) and food (middle panel) and activity levels (lower panel). Water intake = amt. (ml) water consumed in 30-min test; food intake (g; corrected for spillage); activity counts = number of photobeam breaks. *Significantly different from corresponding vehicle (VEH) infusion; $p<0.05$. Open bars: MR; cross-hatched bars: DR.

RESULTS

Of the 24 rats to begin the study, 2 were found to have cannulae placements outside of the MR and were eliminated from further data analysis.

Figure 1 shows the results following vehicle, senktide and muscimol injections on activity, food and water intake. Repeated-measures ANOVAs (region vs. drug) revealed significant drug effects on water intake, $F(4,80) = 12.4$, $p<0.001$, food consumption, $F(4,80) = 30.0$, $p<0.001$, and activity, $F(4,80) = 15.7$, $p<0.001$.

As indicated in the top panel, senktide significantly decreased water consumption when infused into the MR at a dose of 30

pmol. Although 10 and 30 pmol senktide injected into the DR decreased water intake, this did not reach statistical significance ($p>0.05$). In contrast, muscimol significantly elevated water intake following MR infusion.

The middle panel similarly demonstrates that only the highest dose of senktide administered into either the MR or DR significantly diminished the amount of food eaten in the 30-min test session. Infusions of 25 ng of muscimol into the MR and DR significantly increased food intake, although as shown, the effects following MR infusions were much greater. This greater sensitivity of the MR to the hyperphagic effects of muscimol was evidenced by a significant region by drug interaction, $F(4,80) = 2.91$, $p<0.05$.

Depicted in the lower panel of Fig. 1 are the dose-dependent increases in activity induced by infusions of senktide into the MR and DR. Injections of 25 ng of muscimol into the MR but not the DR significantly elevated activity during the 30-min test session. As indicated above for food intake, the greater sensitivity of the MR to the locomotor-stimulating effects of muscimol was evidenced by a significant region by drug interaction, $F(4,80) = 18.8$, $p<0.05$.

DISCUSSION

The control of food and water intake is a complex process which can be manipulated and studied in a variety of ways. Depriving animals of food or water for a period of time will motivate animals to consume. Alternatively, when animals are sated or nondeprived, drinking can be elicited pharmacologically by injections of hypertonic saline, angiotensin II, sodium depletion or by increasing the palatability of the solution [e.g., with sweeteners; as reviewed in Cooper and Turkish (3)]. Likewise, food consumption can be induced pharmacologically with insulin injections, increasing its palatability or by stress (3). Thus, inhibition of either food and water intake is commonly studied in subjects by first inducing consumption by one of these means. Direct chemical or electrical stimulation of CNS regions or neuronal pathways can also be utilized to either stimulate or inhibit consummatory behaviors (3). In the present study, nondeprived rats were used to examine the effects of intra-midbrain raphe injections of the NK-3 agonist senktide on food and water intake. To control for the effects of novelty of the testing apparatus, which can itself inhibit consumption (4), rats were habituated to the testing chamber for 3 days prior to the first test (30 min/day). Intra-raphe injections of senktide, in contrast to the $GABA_A$ agonist muscimol, were found to inhibit food and water intake in the nondeprived state.

Neurokinins have previously been reported to be involved in body fluid regulation. Central (intracerebroventricular; ICV) injections of these peptides inhibit water and salt intake in response to a number of dipsogenic and natriogenic challenges (5, 6, 18, 20, 21, 28). Food intake does not appear to be affected (19,21). Substance P has been demonstrated to be a potent antidipsogenic agent upon central administration (6). Likewise, neurokinin A (NKA) at low doses (62–500 ng, ICV) inhibits drinking produced by subcutaneous injection of hypertonic saline (28). Smaller decreases in water intake were noted for water-deprived rats (28). Higher doses of NKA (1000–2000 ng; ICV) have also been reported to inhibit salt intake following various methods of sodium depletion (18). Senktide has been shown to inhibit salt intake induced by sodium depletion, but has no effect on hypertonic saline-induced drinking (21). Thus, neurokinins appear to play a key role in the regulation of fluid homeostasis although a specific central site has yet to be determined.

Previous studies have demonstrated that intra-raphe injections of the $GABA_A$ agonist muscimol produce dose-dependent increases in food and water intake (10–12) along with a concomitant increase in locomotor activity (29). In the present experiment, 25 ng of muscimol produced significant elevations of food intake following injections into the MR and DR, but only significant increases in water consumption following MR injections. Muscimol was more potent following infusion into the MR than the DR. These results are in agreement with those of Klitenick and Wirtshafter (10).

Both muscimol and senktide produce hyperlocomotion following their injection into the midbrain raphe (26,29). Neurokinin-induced motor stimulation following ICV administration also has been noted (18, 28, 34). The increased locomotion induced by intra-MR senktide suggests that a motor impairment did not cause the inhibition of food and water intake. On the other hand, one could argue that the activation produced by these compounds may interfere with the animal's ability to eat or drink. Stoessl and colleagues (32) have observed that ICV administration of senktide can produce a 5-HT "syndrome" (head weaving and wet-dog shakes). If intra-raphe senktide induced similar stereotypic behaviors, these could interfere with feeding behavior. Thus, one could observe decreases in eating and drinking with increases in photobeam breaks. This is unlikely to have occurred in the present study since we have observed animals in an open field following intra-raphe senktide administration (data not shown) and note increases in locomotion with no evidence of 5-HT "syndrome"-like behaviors. Although one could still argue that the hyperkinesis interferes with consummatory behavior, this is unlikely since muscimol, which also produces hyperlocomotion, stimulates food and water intake.

The opposite effects on consummatory behaviors which intra-raphe senktide and muscimol have might be explained by the fact that the hyperactivity produced by these compounds differs in their anatomical substrates. Although muscimol originally was thought to produce its behavioral affects via 5-HT neurons (29), it has been subsequently demonstrated that 5-HT neurons are not involved (24,36). Likewise, muscimol's effects on food and water intake also do not appear to involve 5-HT neurons (12). We have also demonstrated that intra-MR muscimol injections produce dose-dependent increases in plasma ACTH and corticosterone secretion which are not dependent upon central 5-HT pathways (14). This difference in anatomical substrates could also explain why, in contrast to muscimol, senktide was equipotent following its infusion into the MR and DR.

In contrast, intra-MR 5-HT neurons do mediate the hyperkinetic effects of neurokinin injections (24,26). Enhanced 5-HT release is known to produce an inhibition of eating, presumably by inducing satiety at the level of the hypothalamus (15). The 5-HT releasing agent, fenfluramine, is used clinically as an anorectic agent [see (33)]. However, ingestive behaviors can be increased following peripheral and intra-raphe injections of the $5\text{-}HT_{1A}$ agonist 8-OHDPAT (2,8). This agonist binds to inhibitory somatodendritic autoreceptors to decrease neuronal activity (30). Thus, enhanced 5-HT neurotransmission leads to inhibition of food intake while diminished 5-HT activity stimulates this process. Since the hyperactivity produced by intra-raphe senktide is due to a 5-HT mechanism (24,26), and both enhanced 5-HT neurotransmission and intra-raphe senktide elicit hypophagia, stimulation of neurokinin receptors may produce an increase in the activity of these neurons.

An important consideration in the present study is that senktide inhibits food and water intake in nondeprived subjects and in the absence of any pharmacological or physiological challenges. Other studies have demonstrated that neurokinins inhibit water intake elicited by administration of hypertonic saline, water deprivation, or intracerebral infusion of angiotensin II (5, 6, 20,

28). We have found that in food- or water-deprived (24–48 h) rats, senktide has no effects on food and water intake (data not shown). Other challenges have not been tested. Thus, it would appear that stimulation of NK-3 receptors affects consummatory behaviors in the sated state, but does not affect the animal under pharmacological or motivational stimulation to eat or drink.

The present results indicate that activation of NK-3 receptors by direct administration of senktide in the midbrain raphe is inhibitory to food and water intake. Previous results indicate intra-raphe infusion of NK-1 and NK-2 receptor agonists (substance P and NKA, respectively) also produces hyperlocomotion although senktide is more potent in this regard (25). Whether or not injections of substance P or NKA into the raphe affect consummatory behaviors remains to be explored further.

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