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Microinjection of Neurotensin Into the CNS Induces Hyperdipsia in the Rat[1]

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BAKER, J. D., M. F. HAWKINS, A. A. BAUMEISTER AND M. NAGY. *Microinjection of neurotensin into the CNS induces hyperdipsia in the rat.* PHARMACOL BIOCHEM BEHAV **33**(1) 7–10, 1989.—Neurotensin (NT) is a neuropeptide and putative neurotransmitter that has been shown to exert a variety of effects on digestive and ingestive processes. In order to address the possibility that NT might play a role in the regulation of water intake as well, the peptide was infused into the lateral cerebral ventricle, amygdala, ventral tegmental area, lateral hypothalamus, and preoptic area of the anterior hypothalamus of rats deprived of water for 16 hours. Neurotensin produced a significant and dose-dependent increase in water intake when injected into the ventricular system but had no effect when it was applied to the other brain sites. It was concluded that this peptide may play a physiological role in the control of water ingestion and that central sites of action remain to be determined.

Neurotensin Water intake Drinking Ingestion Neuropeptide

THE tridecapeptide neurotensin (NT) was discovered by Carraway and Leeman (4) while purifying substance P from extracts of bovine hypothalami. Many peripheral and central effects have been ascribed to NT. Systemic application has been reported to produce vasodilation, hyperglycemia, and hypotension (the effect for which the peptide was named) (2–4, 15, 22). In addition to its well-documented central hypothermic (1, 16, 17) and antinociceptive actions (5, 17, 18), CNS injections of NT have been shown to alter digestive processes (19,24) and to decrease food intake (9–11, 14, 19).

Despite a growing body of evidence that implicates NT in the control of digestive and ingestive processes, very little attention has been given to the possibility that this peptide might alter water intake. The most current reviews report no studies of the effects of NT on drinking (17,21) or that CNS injection has no effect on water consumption (12).

A computer-assisted search of the literature revealed only three articles that have addressed this issue. An abstract published by Evered (7) reported that intracerebroventricular (ICV) injection of NT in normally-hydrated rats and pigeons resulted in a dose-dependent increase in water intake. Similarly, Stanley, Hoebel and Leibowitz (26) reported that infusion of NT into the mesenteric vein (1–1000 pmole/kg/min) reduced the latency to drink and increased the amount of water drunk in rats that were not water deprived. Whether this effect was mediated by peripheral or central mechanisms is not known; but the fact that NT seems to cross the blood-brain barrier with difficulty (17) suggests peripheral mediation. Stanley *et al.* (26) also found that bilateral microinjection of NT into the paraventricular nucleus of the hypothalamus of water-deprived animals had no effect on water intake in doses as high as 10 μg/side. In contrast to the dipsogenic effect of ICV NT reported by Evered (7), Yoshikawa (28) found that ICV infusion of NT decreased the water intake of water-deprived rats in a dose-dependent manner (0.5 to 40.0 μg). Yoshikawa's data also differ from those of Stanley *et al.* (26) in that Yoshikawa reported no effect of peripheral (subcutaneous) NT while Stanley *et al.* found intravenous NT to produce an increase in water intake.

In order to determine more completely the role of NT in the control of water intake we have investigated the effects on drinking of lateral ventricular (LV) injections of NT in rats deprived of water for 16 hours. The peptide was also microinjected into the lateral hypothalamus (LH), ventral tegmental area (VTA), amygdala (AMG), and preoptic area of the anterior hypothalamus (POAH) to evaluate potential central sites of action. These sites were selected because they are known to contain NT (6, 12, 13, 21) and have been implicated in the control of drinking behavior (8, 23, 25, 27).

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METHOD

Animals

Male Sprague-Dawley rats (280–320 g) of the Holtzman strain were used. They were housed in individual metal cages at a controlled ambient temperature (22 ± 1°C) with constant light. Purina Rat Chow was available ad lib and water was available for 8 hours from 0800 to 1600 each day.

Surgery and Histology

Stainless steel (22-gauge) guide cannulae were implanted stereotaxically and fitted with 30-gauge obturators to keep the lumen patent. Injection cannulae constructed of 30-gauge, stainless steel, hypodermic tubing were made to extend 1.0 mm below the guide cannulae. The coordinates for the implants (20) were: LV = 0.0 mm anterior to bregma, 1.6 mm lateral to the midsagittal suture, and 3.0 mm ventral to dura; LH = 0.2 mm posterior, 2.0 mm lateral, and 8.0 mm ventral; AMG = 1.2 mm posterior, 3.8 mm lateral, and 8.5 mm ventral, POAH = 2.0 mm anterior, 1.8 mm lateral, and 7.3 mm ventral; VTA = 3.5 mm posterior, 1.0 mm lateral, and 8.0 mm ventral. The animals were implanted under ketamine HCl anesthesia (0.2 mg/g body weight, IM).

After experimental testing, placement of the cannulae was determined by standard histological techniques. The animals were sacrificed with an overdose of chloroform and perfused transcardially with isotonic slaine and 10% formalin. After fixation in formalin for a minimum of 48 hours the brains were blocked at the angle of implant and frozen sections were taken at 40-μm intervals. These were placed on microscope slides, stained with cresyl violet, and examined to verify the location of the implant. Only data from those animals that were accurately implanted were analyzed.

Injections

Neurotensin (Sigma Chemical Co.) was dissolved in sterile normal saline and saline was used for control injections. The obturators were removed, the injector inserted, and the appropriate volume delivered over a 30-second period. The injector was left in place an additional 30 seconds to allow diffusion to occur. Injection volume for the LV group was 5.0 μl infused unilaterally. For site injections into brain tissue a volume of 0.5 μl was infused bilaterally. Previous research (9) has shown that repeated central injections of NT do not alter the peptide's hypophagic action. Therefore, all animals in a group received the same dose of NT on a given day, and the sequence of doses across days was randomly ordered.

Procedures

The rats were acclimated to handling, water deprivation, and the injection procedures before surgery and experimental testing were begun. At 0800 every day each animal was removed from its home cage, weighed, and handled in the manner necessary for injections. After acclimation the animals were randomly assigned to treatment groups and stereotaxic surgery was performed.

The animals were allowed two days of postoperative recovery with free access to food and water before water deprivation was resumed and baseline data collection was begun. During the baseline phase the animals were handled as they were prior to surgery. Additionally, water intake was measured with Richter tubes to the nearest milliliter from 0800 to 1000 each morning at 15, 30, 60, 90, and 120 minutes. Injections of NT were not begun until baseline water intake was stable (i.e., standard errors for group means overlapped for three days). Injection days were spaced 48 hours apart. Any assistants aiding in the monitoring of water intake were naive regarding the experimental conditions.

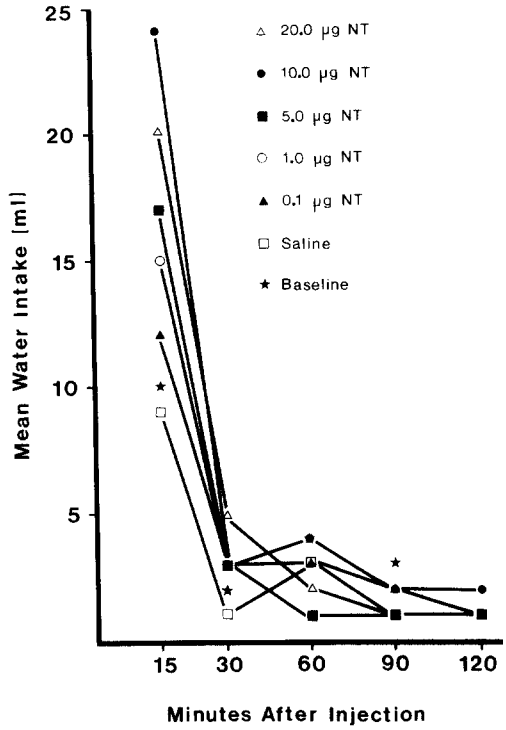

FIG. 1. Mean water intake in milliliters for 120 minutes during the baseline period, and following intracerebroventricular injection of saline and 5 doses of neurotensin.

Data Analysis

The experimental design was within-group and an analysis of variance (ANOVA) for repeated measures was used to examine effects on water intake. The Newman-Keuls test was used for post hoc comparisons. A trend analysis and Pearson product moment correlation coefficients were calculated to evaluate dose-response relationships. Statistical significance is reported for $\alpha = 0.05$.

RESULTS

LV Injections

Water intake for the 6 animals in the LV group is depicted in Fig. 1. Neurotensin resulted in an increase in water intake that was evident at 15 minutes following injection. By 30 minutes after injection, however, water intake returned to control levels. An ANOVA for the first 15 minutes revealed a significant main effect for NT, $F(6,30) = 8.53$. No significant differences among the doses were observed at 30 minutes, $F(6,30) = 1.81$.

The hyperdipsia produced by NT was dose-dependent. Trend analysis of the 15-minute data revealed a significant linear relationship between dose of NT and amount of water consumed, $F(1,25) = 32.30$. Analysis for a quadratic relationship between dose and response was insignificant, $F(1,25) = 0.97$. The correlation between dose of NT and volume of water consumed was 0.65. These data are depicted in Fig. 2.

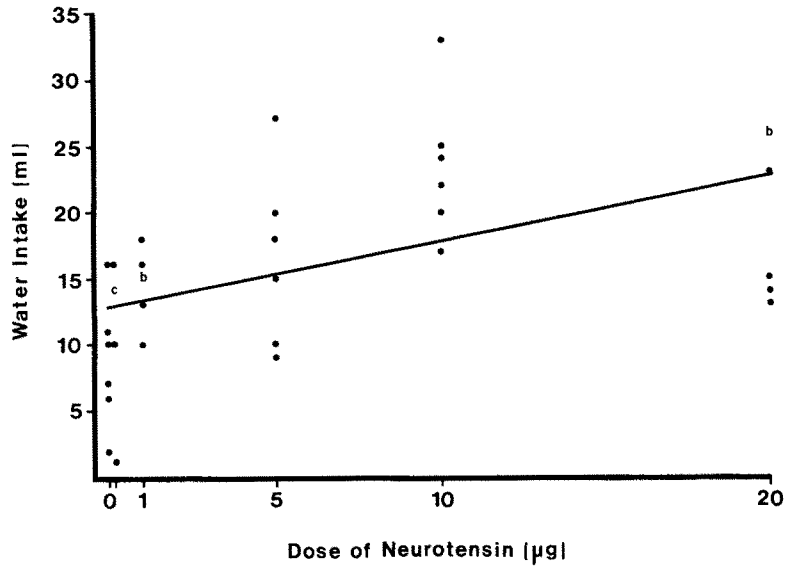

FIG. 2. Dose-response effects of intracerebroventricular saline and neurotensin at 15 minutes following administration (b = 2 coincident data points, c = 3 coincident data points). The 0.1 µg dose of neurotensin is represented, but not labelled, on the abscissa.

Post hoc analysis of the 15-minute data revealed that saline injection did not alter water intake from baseline levels and that the lowest dose of NT did not increase water intake from baseline or saline values. Animals drank significantly more following the 1.0, 5.0, 10.0, and 20.0 µg doses of NT than they did after saline injection. The percentage increases in water intake for these doses compared to saline were 65%, 88%, 167%, and 122% respectively. The hyperdipsia following 10.0 µg and 20.0 µg doses was significantly greater than that following 1.0 µg and 5.0 µg. The effects of the 1.0 µg and 5.0 µg doses did not differ from one another; neither did the effects of the 10.0 µg and 20.0 µg doses.

Site Injections

Mean cumulative water intake at 15 minutes following site injections is presented in Table 1. As was seen with LV injections, vehicle control injections into these brain areas did not change water intake from baseline values. Unlike LV administration, however, NT injected into the LH, AMG, VTA, and POAH in doses as high at 5.0 µg/side had no effect on water intake. In comparison, 5.0 µg of NT injected unilaterally (i.e., half as much as site injections) into the LV increased water intake by 88% relative to control. The ANOVA for the LH group revealed no significant differences among treatment conditions, $F(3,15) = 1.14$. The same result was obtained in the VTA, $F(4,24) = 1.59$, AMG, $F(4,20) = 1.23$, and POAH, $F(3,15) = 0.35$, groups.

DISCUSSION

Injection of NT into the lateral ventricles of 16-hour water-deprived animals resulted in a dose-dependent increase in water intake. This represents the first demonstration of a dose-dependent hyperdipsic effect of NT in deprived rats. These data support Evered's (7) finding of increased spontaneous drinking in rats following ICV NT and suggest that NT may play a physiological role in the control of drinking behavior.

The hyperdipsia reported here and by Evered (7) are not in accord with the hypodipsic action of NT reported by Yoshikawa (28). The reason for this discrepancy is not known; it is doubtful that it is attributable to dose effects as the doses employed here are comparable to those of Yoshikawa.

The fact that NT affects water intake when injected into the cerebral ventricle provides no information regarding central sites of action. Four potential sites were investigated here. The results suggest that the lateral hypothalamus, ventral tegmental area, cortical amygdala, and preoptic area of the anterior hypothalamus are not important receptive areas for neurotensin's control of

TABLE 1

MEAN (±SE) WATER INTAKE AT 15 MINUTES AFTER SITE INJECTION

Site*	Injection	Intake (ml)
LH (6)	baseline	9.3 ± 0.8
LH	saline	9.2 ± 1.4
LH	NT 1.0 µg	11.3 ± 1.6
LH	NT 5.0 µg	8.5 ± 0.9
AMG (6)	baseline	7.3 ± 0.8
AMG	saline	6.7 ± 1.3
AMG	NT 0.1 µg	5.0 ± 2.0
AMG	NT 2.0 µg	10.0 ± 2.5
AMG	NT 5.0 µg	8.2 ± 2.0
VTA (7)	baseline	8.3 ± 0.9
VTA	saline	5.9 ± 1.6
VTA	NT 1.0 µg	8.0 ± 1.5
VTA	NT 2.5 µg	8.7 ± 1.6
VTA	NT 5.0 µg	10.1 ± 0.9
POAH (6)	baseline	11.2 ± 1.1
POAH	saline	10.8 ± 0.7
POAH	NT 1.0 µg	13.0 ± 1.5
POAH	NT 5.0 µg	11.2 ± 3.1

*Value in parentheses represents number of animals in the group.

drinking. Additionally, the findings of Stanley *et al.* (26) suggest that the paraventricular nucleus of the hypothalamus is not an important site of action in the mediation of this effect. Clearly, additional research is required. Some other loci which have been implicated in the control of water intake, are known to contain NT and NT receptors (21), and are therefore worthy of investigation include the zona incerta, subfornical organ, medial hypothalamus, and supraoptic nucleus.

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