Pharmacology Biochemistry and Behavior, Vol. 58, No. 4, pp. 893–898, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00040-3

Effects of Microinjections of Cholecystokinin and Neurotensin into Lateral Hypothalamus and Ventral Mesencephalon on Intracranial Self-Stimulation

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Received 11 March 1996; Revised 18 November 1996; Accepted 4 February 1997

SINGH, J., T. DESIRAJU AND T. R. RAJU. *Effects of microinjections of cholecystokinin and neurotensin into lateral hypothalamus and ventral mesencephalon on intracranial self-stimulation.*PHARMACOL BIOCHEM BEHAV **58**(4) 893–898, 1997.—Changes in intracranial self-stimulation (ICSS) evoked from ventral tegmental area–substantia nigra (VTA-SN) and lateral hypothalamus–medial forebrain bundle (LH-MFB) before and after microinjections of sulfated cholecystokinin octapeptide (CCK-8S) and unsulfated cholecystokinin (CCK-8US), neurotensin tridecapeptide ([D-Tyr^{11}]$NT_{1–13}$ or [DTrp^{11}]$NT_{1–13}$) into either VTA-SN or LH-MFB were assessed. The current intensity was fixed at a level to obtain 60–70% of the maximal asymptotic rate. CCK-8S (0.10 μg/0.5 μl and 0.25 μg/0.5 μl) into VTA-SN resulted in dose-dependent decreases in VTA-SN ICSS of 38–42% and 78–92%, respectively, without affecting the ICSS of LH-MFB. Similar doses of CCK-8S injected into LH-MFB changed neither LH-MFB ICSS nor VTA-SN ICSS. CCK-8Us injected into VTA-SN or LH-MFB had no effect on ICSS in either site. Intra-VTA-SN injections of the neurotensin-1 (NT_1) receptor agonist [DTyr^{11}]$NT_{1–13}$ and the NT_1 receptor antagonist [D-Trp^{11}]$NT_{1–13}$ at doses of 5 μg/0.5 μl and 10 μg/0.5 μl decreased VTA-SN ICSS. NT_1 receptor agonist and antagonist injections did not alter LH-MFB ICSS in any significant manner. Similar injections of these peptides into LH-MFB did not change the responding rates for LH-MFB ICSS or VTA-SN ICSS. Increasing the current intensity reversed the inhibitory effect of CCK-8S and [D-Trp^{11}]$NT_{1–13}$ on VTA-SN ICSS and restored basal preinjection rates of responding. These results suggest that CCK_A and NT_1 receptor mechanisms in the ventral tegmentum in association with dopamine neurotransmission may be important in mediating the rewarding effects of VTA-SN ICSS but not LH-MFB ICSS. © 1997 Elsevier Science Inc.

Cholecystokinin Neurotensin Neuropeptide receptors Intracranial self-stimulation Lateral hypothalamus Medial forebrain bundle Ventral tegmental area Substantia nigra Rat

BEHAVIORAL studies have provided evidence for the role of nonopioid peptides such as cholecystokinin octapeptide (CCK-8), neurotensin (NT) tridecapeptide ($NT\text{-}_{1–13}$) and their receptors in reinforcement (5,6,10–12,29). Considerable differences exist in the effects of CCK an NT on intracranial self-stimulation (ICSS). For example, systemic administration of sulfated CCK-8 increased or decreased ICSS of lateral hypothalamus–medial forebrain bundle (LH-MFB), depending on conditions of food deprivation and satiety, respectively (6). A decrease in LH ICSS also was observed with CCK-8S and its analogue injected intraventricularly, and this effect was blocked by a selective antagonist of CCK_A receptors (14). Microinjections of CCK-8S in the rostral and caudal nucleus accumbens (nACB) decreased and increased LH ICSS, respectively (4,14). Furthermore, injection of proglumide—a nonspecific CCK receptor antagonist—into rostral nACB facilitated ventral tegmental area (VTA) ICSS, and proglumide injection into the caudal nACB attenuated VTA ICSS in a dose-dependent manner (37). CCK-8S injection into VTA attenuated caudal mesencephalic central gray ICSS (29). Bilateral intra-VTA injections of NT produced effects consistent with reward enhancement in the conditioned place preference (11) and self-administration paradigms (10) but decreased responses for food rewards in an operant paradigm (11). NT in-

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jection into VTA lowered the stimulation frequency required to sustain threshold or to produce a half-maximal rate of responding for central gray ICSS (29).

Immunohistochemical evidence supports the existence of a subpopulation of dopaminergic (DA) neurons containing CCK or NT in the VTA and substantia nigra (SN) (29,31). In addition, there are CCK- and NT-containing afferents terminating on SN and VTA neurons (16,20). The presence of CCK and NT in the neurons and local release of those neuropeptides from LH has been demonstrated (13,19,30). The CCK_A receptor subtype occurs in a few localised areas of rat brain including VTA-SN and in gastrointestinal viscera (38). The CCK_A receptor in the LH modulates satiety and potentiates dopamine-induced hypolocomotion (23,24). The CCK_B receptor subtype is found in selective mesocorticolimbic dopaminergic neurons and in spinal cord (38). The CCK_B receptor modulates anxiety, neuroleptic activity and arousal (23,24). NT receptors in the VTA are involved in spontaneous motor behavior and control of behaviors motivated by positive reinforcers (1,19). Dysfunction of the DA–NT interaction is implicated in psychosis (39). NT receptors occur in two forms, the NT_1 and NT_2 receptor subtypes. NT_1 receptors are located in mesencephalic dopaminergic neurons (3,20).

Given the anatomical distribution of these neuropeptides and their receptors in LH and ventral mesencephalon, it is of interest to examine the effects of CCK and NT injection into VTA-SN and into LH on both VTA ICSS and LH ICSS. In the present study, the method for intracerebral neuropeptide microinjection was adapted and combined with multiple electrode placements in reward-relevant areas. In addition, to evaluate the facilitatory or inhibitory effects of peptide injection on ICSS, the electrical stimulation parameters were adjusted to obtain response rates that were 60–70% of the maximal asymptote of the rate–intensity curve.

METHODS

Experimental Subjects

Subjects were Wistar adult male rats weighing 260–320 g at the time of surgery. Following surgery, animals were housed individually in polypropylene cages (22.5 × 35.5 × 15 cm) in a temperature- and humidity-controlled colony with access to lab chow (18% casein, wheat, lipids, vitamins, and minerals; semisynthetic diet) and water. Food and water were available for consumption ad libitum, except during the experimental sessions.

Surgery/Implantation, Behavioral Testing, Microinjections and Histology

Surgery/implantation, behavioral testing, microinjections and histology were conducted as described elsewhere (34). In each rat, following standard stereotaxic procedures, a chronic indwelling bipolar electrode and an insulated cannula/electrode were implanted, one of them in LH-MFB and the other in VTA-SN. The bipolar electrode was made from a pair of insulated stainless steel wires, 220–250 μm, that were fitted to a miniplastic collar. The cannula guide tubing/electrode was a bipolar electrode made of 24-gauge stainless steel tubing to facilitate injection and a 30-gauge wire glued together side by side after insulation. The insulation was done with epoxylite resin (Clark Electromedical Instruments, England). The miniature metal collar was soldered to the guide cannula. The final length was 13 mm, excluding the collar. The predetermined flat skull stereotaxic coordinates were adapted from the atlas of Paxinos and Watson (26): LH-MFB, A-P = −1.8 to −3.3 mm from Bregma: M-L = 1.6–2.0 mm, D-V 8.4 ± 0.2 mm from dura; VTA-SN, A-P = −4.8 to −6.3 mm from Bregma; M-L = 1.1–1.8 mm; D-V = 8.6 ± 0.2 mm from dura. There were two types of placement combinations: (a) LH-MFB bipolar electrode and ipsilateral VTA-SN cannula/electrode (n = 42) and (b) VTA-SN bipolar electrode and ipsilateral LH-MFB cannula/electrode (n = 27). All the implantations were done in the right hemisphere. The electrode/cannula assembly and anchoring screws were secured with acrylic dental cement to the skull. The cannula was sealed and kept patent by insertion of a removable stainless steel stylet when not in use. Contingent to each pedal pressing, a 0.25-s train of sinusoidal electrical stimulation at 50 Hz was delivered. The response/reinforcement data were recorded on an automated digital counter. Reliable, sustainable and reproducible seizure-free rates of ICSS pedal press responses from both sites on a continuous reinforcement schedule were obtained. The stimulating current intensity was changed to obtain the criterion rate without any observable signs of aversiveness, e.g., retreat from pedal, vocalisation, circling, jerking, etc., suggesting accurate electrode placements. The behavioral criterion was a minimum of 50 pedal press responses/min for LH-MFB and a minimum of 80 pedal press responses/min for VTA-SN at the optimum current level for 3 sessions of 30 min each. The optimum current was defined as the minimum current required to obtain consistent responding rates that were 60–70% of the maximum response rates for each rat.

Schedule of Microinjection of the Vehicle and the Neuropeptide and Behavioral Testing

The dose–response and time–effect patterns of each neuropeptide were assessed in initial experiments for selecting optimal doses and optimal timings for further experimentation.

Vehicle. Vehicle injections were administered 10 min before ICSS testing. The ICSS testing sessions lasted 15 min (V), which was followed by a 10-min ICSS-free period. Testing of ICSS responses from the other site was done for 15 min (B). After this testing, an interval of 30 min without ICSS testing was allowed before neuropeptide injection.

Neuropeptide. Injection of the neuropeptide was followed by a predetermined time interval based on time–effect patterns observed in preliminary experiments. Testing of ICSS responses from the first injection site was carried out for 15 min (D). After a 10-min interval, testing of ICSS responses from the other site was conducted for 15 min (A). In half of the rats, the testing was done in reverse order to avoid a time delay and to have a balanced experimental design. Animals were tested with vehicle and one dose of neuropeptide on a given day. Tests with different neuropeptides were separated by at least 48 h.

Materials

CCK-8S (sulfated [Tyr SO_3H^{27}] cholecystokinin$_{26–33}$), CCK-8US (unsulfated [Try_{27}] cholecystokinin$_{26–33}$), [D-Tyr^{11}] $NT_{1–13}$ and [D-Trp_{11}]$NT_{1–13}$ were obtained from Sigma (St. Louis, MO). These peptides were dissolved in artificial cerebrospinal fluid (aCSF). The aCSF was prepared by dissolving 126 mM NaCl, 27.5 mM $NaHCO_3$, 2.4 mM KCl, 1.1 mM $CaCl_2$. $2H_2O$, 0.8 mM $MgCl_2$. $6H_2O$, 5 mM Na_2CO_3 and 5.9 mM glucose in double-distilled H_2O (pH 7.2) and then passed through filter paper (0.45 μm). The working solutions of 20 μg/μl were stored in separate polypropylene vials at −4°C and diluted when required before the experiments.

The CCK_A receptor subtype exhibits a high affinity for CCK-8S but not for CCK-8US (15). The CCK_B receptor subtype binds both CCK-8S and CCK-8US with approximately equimolar affinity (23,38). The [D-Tyr[11]] NT_{1-13} is an NT_1 receptor agonist, and [D-Trp[11]] NT_{1-13} is an NT_1 receptor antagonist.

Statistical Analysis

The results were analysed using two-way analysis of variance (ANOVA). Following significance in the overall ANOVA, matched-paired *t*-tests (two-tailed) were applied for the comparison of response rates following vehicle (control) and neuropeptide (experimental) injections and also before and after neuropeptide microinjection.

RESULTS

CCK Receptor Agonists

Microinjection of CCK-8S into VTA-SN in doses of 0.10 μg/0.5 μl (0.16 nM) and 0.25 μg/0.5 μl (0.44 nM) decreased VTA-SN ICSS by 38–42% ($t = 3.81$, $p < 0.01$) for the 0–16-nM dose and 78–92% ($t_6 = 12.56$, $p < 0.001$) for the 0.44–nM dose as compared with vehicle injection. LH-MFB ICSS was unaffected (Fig. 1a, left panel). CCK-8S (0.16 nM, 0.44 nM) injected into LH-MFB did not produce any significant change in either LH-MFB ICSS (Fig. 1b, left panel).

In contrast, when similar doses of CCK-8US [0.10 μg/0.5 μl (0.18 nM) and 0.25 μg/0.5 μl (0.45 nM)] were injected into either VTA-SN or LH-MFB, there was no effect on ICSS responding from the site of injection, or from the other ICSS site (Fig. 1a and 1b, right panel).

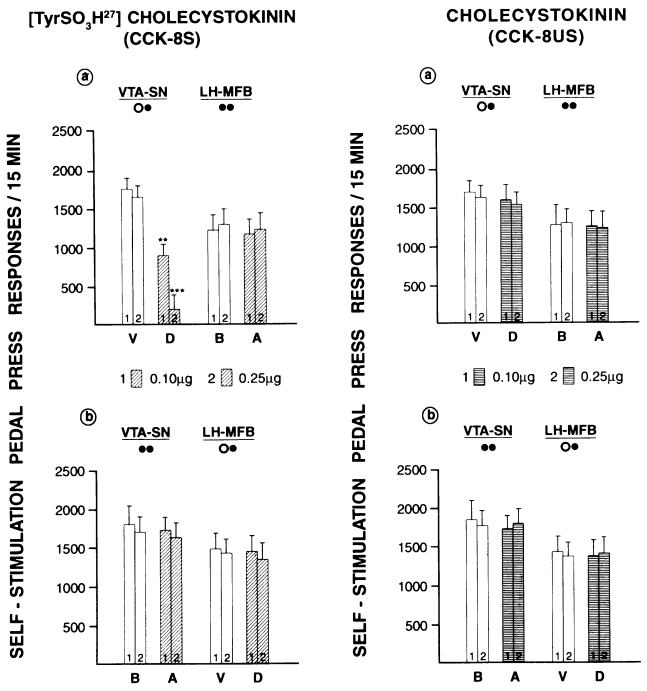

FIG. 1. Effects of microinjection of (a) CCK-8S (0.10 μg and 0.25 μg/0.5 μl) into either VTA-SN (upper left panel) or into LH-MFB (lower left panel) and (b) CCK-8US (0.10 μg or 0.25 μg/0.5 μl) into either VTA-SN (upper right panel) or into LH-MFB (lower right panel) on the ICSS derived from the injected site (D) as compared with the vehicle (V) and also on the ICSS from the alternate site before (B) and after (A) the drug injection. Seven animals were tested for each group except the intra-VTA-SN injection site, for which the number of animals was 8. Cannula/electrodes and bipolar electrodes are represented by an open circle along with a small filled circle and by a pair of small filled circles, respectively. Explanations of the schedule of behavioral testing and the sequence and timing of the events denoted by V, D, B, and A are given in the Methods section. $*p < 0.05$ or 0.02, $**p < 0.01$; $***p < 0.001$ for matched-paired *t*-tests (two-tailed); the comparisons are between D and V and between A and B. Bar on each histogram represent standard deviation.

NT Receptor Agonist and Antagonist

Two doses [5 μg/0.5 μl (5.96 nM) and 10 μg/0.5 μl (11.92 nM)] of [D-Tyr[11]] NT were tested. Injection of the higher dose into VTA-SN decreased VTA-SN ICSS by 37–53% ($t = 3.18$, $p < 0.02$), whereas LH-MFB ICSS remained unaltered (Fig. 2a, left panel. The injection of these two doses of [D-Tyr[11]] NT into LH-MFB did not produce any change in either LH-MFB ICSS or VTA-SN ICSS (Fig. 2b, left panel).

Intra-VTA-SN injection of 5 μg/0.5 μl (5.88 nM) and 10 μg/0.5 μl (11.76 nM) of [D-Trp[11]] NT attenuated VTA-SN ICSS by 54–63% ($t = 4.26$, $p < 0.01$) and 72–78% ($t = 7.29$, $p < 0.001$), respectively. LH-MFB ICSS was unaltered (Fig. 2a, right panel). The same doses injected into LH-MFB produced no change in either LH-MFB ICSS or VTA-SN ICSS (Fig. 2b, right panel).

Restoration of Preinjection Level of ICSS After Microinjection of Neuropeptides by Increasing the Current Intensity of Electrical Stimulation

To determine whether increasing the current intensity would restore basal preinjection rates of ICSS responding af-

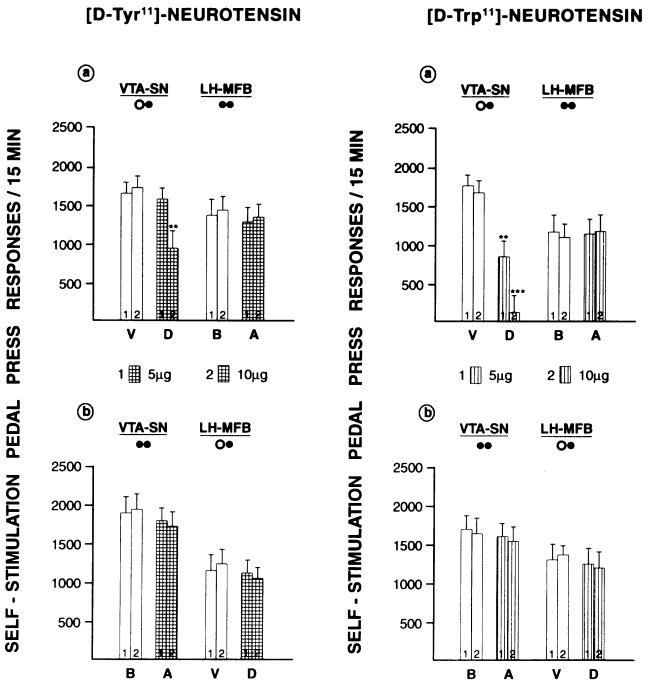

FIG. 2. Effects of microinjections of (a) [D-Tyr[11]] NT (5 μg and 10 μg/0.5 μl) into either VTA-SN (upper left panel) or into LH-MFB (lower left panel) and at (b) [D-Trp[11]] NT (5 μg and 10 μg/0.5 μl) into either VTA-SN (upper right panel) or into LH-MFB (lower right panel) on the ICSS. Ten animals were tested for each group. See Fig. 1 for legend.

ter CCK-8S or [D-Trp[11]] NT injection, the intensity of current was raised in a stepwise manner. After treatment with 0.10 μg/0.5 μl of CCK-8S, a 38–42% suppression of VTA-SN ICSS was observed, and 85–90% more current was required to restore basal responding. After treatment with 0.25 μg/0.5 μl of CCK-8S, a 78–92% suppression was observed, and 180–200% more current was required to restore basal responding (Fig. 3a). Similarly, 70% and 180% more current intensity were required to overcome the inhibitory effects of 5 μg/0.5 μl and 10 μg/0.5 μl of [D-Trp[11]] NT, respectively (Fig. 3b).

Figure 4 shows the histologically verified sites of placement of cannula electrode and bipolar electrode on the coronal sections of the plates of Paxinos and Watson's rat atlas (26). The analysis revealed that tips of cannula electrode were found to lie within a region that has been shown to contain CCK and NT receptor binding sites (3,16–18,31).

DISCUSSION

Intra-VTA-SN injection of CCK-8S attenuated VTA-SN ICSS in a dose-dependent manner. CCK-8US did not change ICSS in any significant manner when it was injected into either VTA-SN or LH-MFB. Because CCK-8US is a selective agonist of the CCK_B receptor subtype, whereas CCK-8S binds to both CCK_A and CCK_B receptors, the observed effects may be attributed to mediation of ICSS by CCK_A receptors. This result is a direct demonstration of alteration of VTA-SN ICSS through CCK_A receptors within the VTA-SN, in contrast to the previous reports that have shown effects of VTA or LH ICSS following microinjections of CCK-8S in rostral or caudal parts of nACB (4,5,25,36,37).

Because intra-LH injections of CCK-8S did not alter VTA-SN ICSS and intra-VTA-SN injections did not alter LH-MFB ICSS, it is reasonable to hypothesize that CCK action is restricted to the VTA-SN substrates. This finding is in agreement with electrophysiological studies that have shown no effect of iontophoretic application of CCK-8S on neurons of the LH (18). CCK-8S produces excitatory effects of the majority of tested SN and VTA neurons and inhibitory effects on a subset of VTA neurons (8,21,35). Hence, it is reasonable to assume that intra-VTA-SN injections of CCK-8S could have preferentially targeted DA-CCK neurons that are inhibited by CCK-8S. It is also possible that attenuation of VTA-SN ICSS may potentiate negative feedback inhibition mediated by DA autoreceptors. The results showing no effect on LH-MFB ICSS and VTA-SN ICSS following intra-LH injections of CCK-8S rule out the possibility that the observed effects could be due to satiety (6).

Intra-VTA-SN injections of an NT_1 receptor agonist and an NT_1 receptor antagonist attenuated VTA-SN ICSS without influencing the LH-MFB ICSS. Further, injection of these neuropeptides into LH-MFB caused no effect on LH-MFB ICSS or on VTA-SN ICSS.

Light microscopic autoradiographic investigations have shown that NT receptors are concentrated mainly over the perikarya and dendrites of DA neurons in substantia nigra pars compacta (SNC) and VTA (28). High densities of NT binding sites also have been shown in different cellular structures (i.e., cell bodies and terminals) in SNC and VTA (3). Microinjection of NT, but not CCK, into VTA increases the concentration of DA and its metabolites in posterior nACB (22). Electrophysiological studies have indicated that iontophoretic application of NT in SN and VTA increases the firing rate of subset of DA

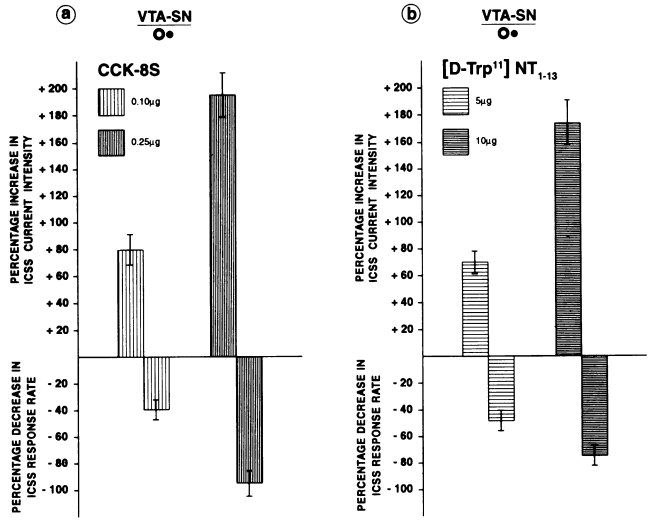

FIG. 3. Percentage increase in self-stimulation current intensity to counteract the inhibitory effect of (a) CCK-8S (0.10 μg and 0.25 μg/0.5 μl) and (b) [D-Trp[11]] NT (5 μg and 10 μg/0.5 μl) and restore the preinjection level of responding. Seven animals were tested for each group.

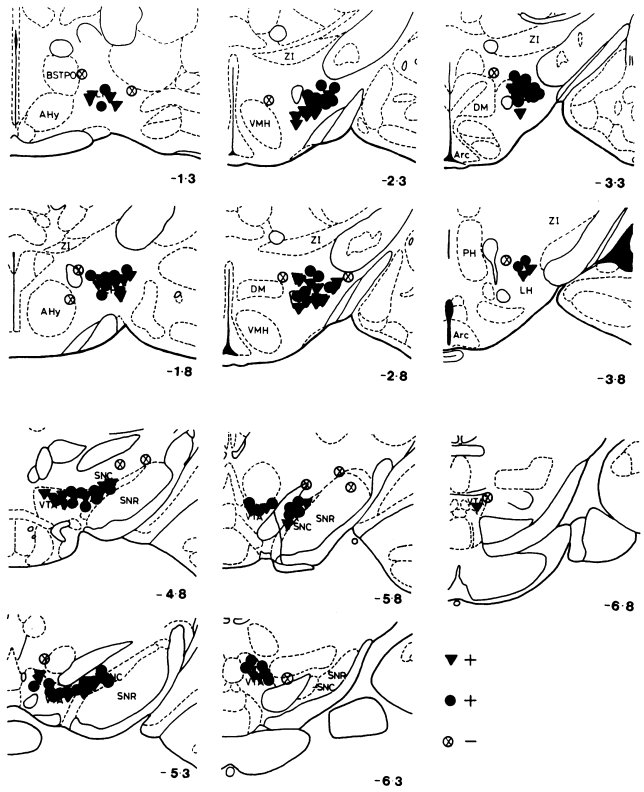

FIG. 4. The histologically verified sites of placement of cannula/electrodes ($n = 62$) and bipolar electrodes ($n = 62$) are represented by filled triangles and circles, respectively, on the coronal sections of plates from the atlas of Paxinos and Watson (26). Data from 7 rats could not be verified. Circles filled with Xs indicate the sites where bipolar electrode or cannula/electrodes either did not produce criterion level responding rates or were negative for self-stimulation ($n = 17$).

neurons (27,32,33). NT also attenuates the inhibitory action of DA or a D_2 receptor agonist (quinpirole) on neurons in the ventral mesencephalon (33), which would imply that NT action is like that of D_2 receptor antagonists (antidopaminergic) with respect to its effect on DA neuron firing and its effect in terminal fields in the dorsomedial nACB. If so, then NT antagonists would behave like dopaminomimetics. Thus, NT microinjection into VTA-SN could have caused an imbalance in the firing of DA and non-DA neurons that contain NT. Such an alternation of VTA-SN neurons may have resulted in the disruption of ICSS derived from VTA-SN. In addition, a similar imbalance in the firing of VTA-SN neurons, but in the opposite direction, can be suggested as an explanation for the suppression of ICSS of VTA-SN with the NT receptor antagonist.

High densities of NT receptors are present on NT-containing afferents in the VTA (3). It has been suggested that released NT can diffuse and generate an extracellular NT–DA complex or act on NT receptors of extrasynaptic regions in a paracrine manner (3). This suggestion would mean that the function of NT in VTA-SN is very different from the way other neurotransmitters and neuropeptides operate.

Bilateral intra-VTA injections of NT produce an amphetaminelike increase in motor activity after a period of 40 min in rats (21). Because the ICSS responses of VTA-SN were decreased by intra-VTA-SN injections and the ICSS responses from the LH were not affected, the change in motor activity, if any, may not have confounded the results. Because injection of these neuropeptides into VTA-SN did not alter LH-MFB ICSS, synaptic mechanisms within the two regions are probably independent of each other (33).

The observation of self-administration of NT into VTA (10) and the suppression of VTA ICSS following intra-VTA administration of NT appear to be contradictory and paradoxical. Nevertheless, it is not difficult to reconcile these results. as the same has been observed with apomorphine, which is self-administered in rat (39) but suppresses VTA-SN ICSS when infused into that site (2). The effect of apomorphine is dependent on dose; thus, high and low doses have opposite effects depending on their ability to differentially stimulate DA receptor subtypes (7). However, it does not necessarily follow that similar mechanisms are valid for NT effects. Both the agonist and antagonist of NT_1 receptors administered into VTA-SN caused reduction of VTA-SN ICSS. The similarity in the agonist and antagonist effects is not difficult to explain. For example, similar effects on certain responses have been observed [i.e., suppression of ICSS following the administration of apomorphine, a DA receptor agonist (2,7) and pimozide, a DA receptor antagonist (9)]. It is reasonable to assume that one or both of the compounds may exhibit some sort of U-shaped dose–response curve. The failure to observe a facilitatory effect with the doses of NT_1 agonist used in the present study suggests that a detailed analysis of dose–response and time–effect must be undertaken with more specific receptor ligands.

Taken together, the results of the present study and other published reports provide support for the mediation by CCK_A and NT_1 receptors of the rewarding effect of ventral mesencephalon self-stimulation but not of lateral hypothalamic self-stimulation.

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