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Effects of Centrally Administered Neuropeptides on Discriminative Stimulus Properties of Cocaine in the Rat

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UKAI, M., E. MORI AND T. KAMEYAMA. *Effects of centrally administered neuropeptides on discriminative stimulus properties of cocaine in the rat.* PHARMACOL BIOCHEM BEHAV **51**(4) 705–708, 1995. – The present study was designed to investigate the effects of centrally administered neuropeptides on the discriminative stimulus properties of cocaine in the rat. Rats were trained to discriminate 10.0 mg/kg of cocaine from vehicle in a shock avoidance paradigm. The μ-selective opioid agonist [D-Ala^2,*N*MePhe^4,Gly-ol]enkephalin (DAMGO) (0.03–0.3 μg, ICV) or the κ-selective opioid agonist dynorphin A-(1–13) (1.0–10.0 μg, ICV) did not generalize to cocaine cue, although the δ-selective opioid agonist [D-Pen^2,L-Pen^5]enkephalin (DPLPE) (10.0 μg, ICV) reportedly generalizes to it through the mediation of δ-opioid receptors. Thyrotropin-releasing hormone (10.0–56.0 μg, ICV), somatostatin (0.3–3.0 μg, ICV), substance P (3.0–17.5 μg, ICV), or neurotensin (3.0–17.5 μg, ICV) did not produce any stimulus effects in common with cocaine. It appears that neuropeptides other than the δ-selective opioid do not play a major role in the discriminative stimulus properties of cocaine.

Cocaine Neuropeptides Opioid peptides Discriminative stimulus Properties Rat

DRUG discrimination procedure has been well established to clarify the subjective effects of drugs related to their abuse (8). Cocaine is a psychomotor stimulant possessing abuse potential (10), whereas the discriminative stimulus effects of cocaine are reportedly mediated through the stimulation of both dopamine D_1 and D_2 receptors in the brain (4,11,20,24,27).

On the other hand, there are different findings about the relationship between cocaine and opioid systems. Recent evidence demonstrates that the density of [^{3}H]naloxone binding increases in a number of brain regions following gestational exposure to cocaine (5). Buprenorphine, a mixed opioid agonist-antagonist, suppresses cocaine self-administration in monkeys (13). Naloxone antagonizes the locomotor-activating effect of cocaine and attenuates cocaine-induced conditioned place preference in rats (9). Moreover, the μ-opioid agonist methadone, as well as buprenorphine, reportedly potentiates cocaine-induced conditioned place preference in rats (2,3) and enhances the discriminative stimulus effects of cocaine, although none of the μ-opioid agonists themselves produce cocaine-appropriate responding in monkeys (19). However, a certain dose of morphine partially attenuates cocaine cue in rats trained to discriminate between cocaine and vehicle, although it does not produce cocaine-appropriate responding (6). κ-Opioid agonists reduce the discriminative stimulus effects of cocaine, although none of the κ-opioid agonists themselves produce cocaine-appropriate responding in monkeys (19). Further, systemic administration of dynorphin A-(1–13), a κ-selective opioid agonist, inhibits different behavioral responses induced by cocaine in mice (25). In contrast, the δ-selective opioid agonist DPLPE reportedly generalizes to cocaine cue, whereas the cocaine-like stimulus effects of DPLPE are almost completely reversed by the δ-selective opioid antagonist naltrindole (23). However, the effects of highly μ- and δ-selective opioid agonists on the discriminative stimulus effects of cocaine have not been determined in detail.

In addition to opioid agonists, there are some findings that neuropeptides interact with dopamine systems. The hypermotility induced by thyrotropin-releasing hormone (TRH) is markedly blocked by the dopamine antagonists haloperidol and pimozide (15). Cysteamine, a selective depletor of somatostatin, also decreases apomorphine-induced stereotypy and amphetamine-induced hypermotility (12). There is evi-

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dence in rats that substance P increases locomotor activity by altering activity in dopamine systems (1). Moreover, neurotensin increases the formation of dopa and accelerates the disappearance of dopamine (7).

The present study was designed to further characterize the discriminative stimulus effects of cocaine with reference to neuropeptides using two-choice discrete-trial avoidance paradigm. Thus, rats were trained to discriminate cocaine (10.0 mg/kg) from vehicle and, subsequently, generalization tests with central administration of highly selective opioid agonists such as [D-Ala^2,*N*MePhe^4,Gly-ol]enkephalin (DAMGO) and dynorphin A-(1-13), TRH, somatostatin, substance P, and neurotensin were carried out.

METHOD

Animals

The subjects were male Fischer-derived rats (Nihon Charles River Co., Ltd., Atsugi, Kanagawa, Japan) weighing 210-230 g at the start of discrimination training. The rats were housed in a ventilated colony room where they had continuous access to food and water. The lights in the room were illuminated between 0800 and 2000 h.

Procedure

Rats were trained to discriminate 10.0 mg/kg of cocaine from vehicle in a two-choice discrete-trial avoidance paradigm (22,24). The onset of a trial was signalled by the simultaneous illumination of the house light and the presentation of white noise. At this time, the rat was required to press the "starting" lever mounted in one wall of the test chamber and then to press one of the two "choice" levers mounted in the opposite wall. The first starting response of the trial terminated the white noise and the appropriate choice response extinguished the house light and ended the trial. Beginning 5.0 s after the onset of the trial, 2.0-3.0-mA shock was delivered to the grid floor of the chamber every 3.0 s in 0.5-1.0-s pulse until the two-response chain was completed. The intertrial interval was 50 s during which time the chamber was dimly illuminated by a red light. Experimental sessions ended after 21 trials or 30 min, whichever came first. The first trial of each session was considered a "warm-up" and was excluded from the data analysis. Training sessions were conducted 6 days/week. Either cocaine (10.0 mg/kg) or its vehicle was injected IP 30 min before each training session. Training continued until rats could reliably complete at least 18 of 20 trials (i.e., 90% exclusive of the first trial) on the appropriate choice lever under both conditions. Once this criterion was met, a cannula was implanted in the lateral cerebral ventricle of the rats. Rats were anesthetized with sodium pentobarbital (50.0 mg/kg, IP). When necessary, chloral hydrate (200.0 mg/kg, IP) was used as an adjunct to anesthesia. The rats were placed in a stereotaxic instrument (Narishige Inc., Tokyo, Japan) and a 22-ga stainless steel guide cannula (No. C313G, Neuroscience, Inc., Tokyo, Japan) was inserted into the left lateral ventricle (AP, −0.8 mm relative to bregma; L, +1.4 mm; V, −3.5 mm relative to dura)(16). After recovery from surgery, training sessions were resumed until stimulus control of behavior was reestablished (i.e., 18 of 20 trials completed on the appropriate choice lever). Drug test sessions were conducted provided the rats satisfied the performance criterion in two consecutive training sessions. During test sessions, both choice levers were activated so that a response on either choice lever after the starting response terminated the trial. Test sessions and training sessions were identical in all other aspects.

Drugs and Treatments

The following drugs were used: cocaine hydrochloride (Shionogi Co., Ltd., Osaka, Japan); DAMGO (Peninsula Laboratories, Inc., Belmont, CA, USA); dynorphin A-(1-13), TRH, somatostatin, substance P, and neurotensin (Peptide Institute, Inc., Osaka, Japan). Doses of the drugs were expressed in terms of the free base. Cocaine was dissolved in isotonic saline (0.9% NaCl, pH 7.5). The peptides were dissolved in sterile isotonic saline (Otsuka Co., Ltd., Tokyo, Japan). Drugs were given 30 min before the session. Cocaine was given IP, whereas the others were given ICV. Cocaine was injected in a volume of 1.0 ml/kg body weight. Intracerebroventricular injections were made by first backloading saline or the drug solutions into a 28-ga stainless steel injection cannula (C313I, Bioresearch Center, Inc., Nagoya, Japan) connected to microsyringe by PE-20 tube. A volume of 5.0 μl/rat was then administered at a rate of 1.0 μl/30 s. The injection cannula was left in place for at least 1 min after injection.

Data Analysis

The data were analyzed in terms of the number of trials completed on the cocaine-appropriate lever. Trials completed on the vehicle-appropriate lever were recorded but are not shown in the figures. All of the animals used completed all trials of every session. A dose of test drug was considered to produce discriminative stimulus effects comparable to those produced by the training dose (10.0 mg/kg) of cocaine if a rat completed at least 18 of 20 trials on the cocaine-appropriate lever.

RESULTS

The μ-selective opioid agonist DAMGO (0.03 μg) produced vehicle-appropriate responding, and increasing the dose to 0.3

TABLE 1

DISCRIMINATIVE STIMULUS EFFECTS OF GRADED DOSES OF NEUROPEPTIDES IN RATS TRAINED TO DISCRIMINATE 10.0 MG/KG OF COCAINE FROM VEHICLE

Neuropeptides	Dose (μg)	Trials to Cocaine Lever*
DAMGO	0.03	0.7 ± 0.5
	0.1	2.8 ± 1.9
	0.3	3.5 ± 1.3
Dynorphin A-(1-13)	1.0	0.0 ± 0.0
	3.0	0.5 ± 0.5
	10.0	0.3 ± 0.2
TRH	10.0	2.0 ± 1.3
	30.0	1.0 ± 0.5
	56.0	2.0 ± 1.2
Somatostatin	0.3	0.3 ± 0.3
	1.0	1.3 ± 1.3
	3.0	2.0 ± 2.0
Substance P	3.0	1.0 ± 0.5
	10.0	2.0 ± 1.3
	17.5	0.3 ± 0.3
Neurotensin	3.0	0.3 ± 0.3
	10.0	0.5 ± 0.3
	17.5	0.3 ± 0.3

*Data are reported as the mean number of trials (± SE) of a 20-trial session completed on the cocaine-appropriate lever. The remaining trials were completed on the vehicle-appropriate lever and are not shown. The criteria for cocaine- and vehicle-appropriate responding are established at 18 and 2 responses, respectively. n = 4-5.

μg also produced almost vehicle-appropriate responding, whereas a higher dose (1.0 μg) of DAMGO disrupted lever-pressing behavior. The κ-selective opioid agonist dynorphin A-(1-13) produced only vehicle-appropriate responding across the range of doses from 1.0 to 10.0 μg, whereas a higher dose (30.0 μg) of dynorphin A-(1-13) disrupted lever-pressing behavior. TRH (10.0-56.0 μg), somatostatin (0.3-3.0 μg), substance P (3.0-17.5 μg), or neurotensin (3.0-17.5 μg) did not produce cocaine-appropriate responding, whereas higher doses of these neuropeptides disrupted lever-pressing behavior (Table 1). In addition, all of the data shown in Table 1 were obtained from rats which completed the session within 30 min.

DISCUSSION

These data should demonstrate the accuracy and effectiveness of the drug discrimination procedure employed in the present study. For example, the dopamine reuptake inhibitor GBR 12909, methamphetamine and apomorphine produce stimulus effects in common with cocaine. Haloperidol combined with SCH23390, a dopamine D_1 receptor antagonist almost completely suppressed the discriminative stimulus effects of cocaine (24).

The δ-selective opioid antagonist naltrindole has been reported to block cocaine-induced conditioned place preference (14). Moreover, the δ-selective opioid agonist DPLPE has been demonstrated to generalize to cocaine cue, whereas the cocaine-like stimulus effects of DPLPE are almost completely reversed by naltrindole. These findings indicate that δ-opioid receptors play a major role in the discriminative stimulus effects of cocaine.

The present result showing that the μ-selective opioid agonist DAMGO did not generalize to cocaine cue in rats is consistent with that reported previously, using μ-opioid agonists such as morphine, levorphanol, and methadone in rats and monkeys (6,19). Because DAMGO provides an order of magnitude increase in opioid receptor selectivity compared with morphine, levorphanol, and methadone, it is further evident that μ-opioid receptors do not play a major role in the stilmulus effects of cocaine. Morphine, levorphanol, and methadone enhance the discriminative stimulus effects of cocaine in monkeys (19). Methadone also potentiates cocaine-induced conditioned place preference in rats (2). These results show that the μ-opioid agonists themselves do not generalize to cocaine cue, but these could play a facilitating role in cocaine abuse.

The present results indicating that the κ-selective opioid agonist dynorphin A-(1-13) did not generalize to cocaine cue in rats are consistent with those by using opioid agonists such as CI 977 and U-50,488 in monkeys (19). These results suggest that κ-opioid receptors do not mediate the stimulus effects of cocaine. The κ-opioid agonists, in particular, suppress the discriminative stimulus effects of cocaine in monkeys (19). Further, systemic administration of dynorphin A-(1-13) inhibits different behavioral responses induced by cocaine in mice (25). These results indicate that the κ-opioid agonists themselves do not generalize to cocaine cue, but these could suppress cocaine abuse, although there seems no direct evidence demonstrating the role of κ-opioid receptors in the reinforcing effects of cocaine.

Although neuropharmacological investigations suggest that various biogenic amines play an important role in rewarding or reinforcing properties of cocaine, the inhibition of dopamine reuptake by binding to dopamine transporters seems to underlie the behavioral efects of cocaine (17). The discriminative stimulus effects of cocaine are reportedly mediated through the stimulation of both D_1 and D_2 dopamine receptors (4,11,20,24,27). It has been reported that DAMGO and the δ-selective opioid agonist [D-Pen^2,D-Pen^5]enkephalin enhance dopamine release in the brain, and conversely, the κ-selective opioid agonist E-2078 attenuates it (18). However, neither DAMGO nor dynorphin A-(1-13), except for DPLPE, generalized to cocaine cue. Thus, the dopamine neurons in direct connection with opioid receptors may not fully contribute to the stimulus effects of cocaine.

There is a possibility that several neuropeptides interact with dopamine systems. The hypermotility induced by TRH is markedly blocked by the dopamine antagonists haloperidol and pimozide (15), and there are several similarities between the behavioral effects of TRH and *d*-amphetamine (26). Cysteamine, a selective depletor of somatostatin, also decreases apomorphine-induced stereotypy and amphetamine-induced locomotor activity (12). In the substantia nigra and ventral tegmental area, synaptic contacts have been found between dopaminergic neuronal cell bodies and substance P-containing nerve terminals (21), and there is evidence in rats that substance P increases motor behavior by altering the activity of dopamine systems (1). Moreover, neurotensin increases the formation of dopa and accelerates the disappearance of dopamine (7). In this study, however, these neuropeptides examined did not produce cocaine-appropriate responding, therefore suggesting that cocaine does not produce stimulus effects in common with TRH, somatostatin, substance P, and neurotensin. These data support our previous findings that the discriminative stimulus effects of cocaine are mediated through the activation of dopamine (D_1 plus D_2) and δ-opioid receptors (23,24).

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