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Asymmetric Behavior Induced by Enkephalinergic Agents in the Basal Ganglia

CHANGIZ GEULA[1] AND DAVID ASDOURIAN

Biopsychology Laboratory, Department of Psychology, Wayne State University, Detroit, MI 48202

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GEULA, C. AND D. ASDOURIAN. *Asymmetric behavior induced by enkephalinergic agents in the basal ganglia.* PHARMACOL BIOCHEM BEHAV **23**(2) 207–213, 1985.—The caudate-putamen (CDp) and the globus pallidus (GP) are sites rich in both leucine (LEU) and methionine-enkephalin (MET-ENK) and in ENK receptors. Since chemical and electrolytic lesions of the CDp and GP result in a reduction in ENKs and their receptors and in motor asymmetry, there may be a role for CDp and GP ENKs in rotational behavior and bodily asymmetry. To test this possibility, various doses of D-ALA-2-LEU-ENK, D-ALA-2-MET-ENK, naloxone and naltrexone were injected into the CDp and GP through chronically implanted cannulae. The injections of MET and LEU-ENK caused dose-dependent ipsiversive rotations while injections of naloxone and naltrexone caused contraversive rotations. All of the drug injections also caused bodily asymmetries which were in the same direction as the circling. Intraperitoneal injections of naloxone dose-dependently blocked the rotational behavior induced by the most effective dose of the ENKs used. ENK injections into sites adjacent to the CDp and GP (i.e., cortex, nucleus accumbens and the region bordering the bed nucleus of the stria terminalis and the bed nucleus of anterior commissure) failed to produce any significant circling. These results clearly suggest that CDp and GP ENKs cause ipsiversive rotational behavior and bodily asymmetry and must be considered as one element of the control exerted by the basal ganglia over the motor system.

Enkephalins Rotational behavior Bodily asymmetry Caudate-putamen Globus pallidus

FOLLOWING the proposal by Ungerstedt [48] that rats will circle away from the side of greater striatal dopamine (DA) receptor activity, a considerable body of research has emerged devoted to clarifying the anatomical, neurophysiological and pharmacological bases of the circuitry involved in controlling rotational behavior and motor asymmetry [35]. A major line of research in this area has concentrated on the contribution of various neurochemicals contained within the basal ganglia to motor asymmetry. One group of these neurochemicals which are present in high concentrations within the basal ganglia but have received little attention with regard to their role in rotational behavior and bodily asymmetry are the enkephalins [42].

The caudate-putamen (CDp) and the globus pallidus (GP) have the highest concentrations of brain leucine (LEU) and methionine-enkephalin (MET-ENK) and are rich in ENK receptors [2, 5, 13, 14, 22, 34, 40, 43, 47]. In the CDp, ENKs are localized within cell bodies and terminals of interneurons and within projection neurons [43] while within the GP they are localized within terminals which belong to a long striopallidal projection system [3, 4, 9, 11, 12, 14, 27, 44]. In both the CDp and GP ENKs are localized in vesicles at axonal endings [14,46], are released through electrical stimulation [3, 5, 52], and upon release cause hyperpolarization of neurons [15, 23, 24, 31, 33, 39, 45].

Within the CDp, ENKs interact with the nigrostriatal DA system. Intracranial and intraperitoneal (IP) injections of opiates, including LEU and MET-ENK, result in a dose-dependent increase in the rate of DA turnover [1,28] and DA release [6,7] in the striatum. This increased DA release and turnover does not necessarily result in facilitation of DA transmission since recent evidence has shown opiates to inhibit adenylate cyclase activity [25] and adenylate cyclase induced phosphorylation of membrane proteins [8].

The behavioral effects of opiates support the possibility that opiates in the striatum interfere with DA transmission [17,18]. Intraperitoneal and intracranial administration of opioid agents in a variety of species causes hypokenesia, catatonia and muscular rigidity. These motor effects are also produced through neuroleptic administration and are easily inhibited by application of DA agonists [38]. In addition, narcotic antagonists significantly enhance the effects of DA mimicking drugs [21]. For example, the hyperthermic effects of apomorphine are significantly enhanced through naloxone administration [36] and both naloxone and naltrexone significantly potentiate the anticatatonic activity of L-dopa [30].

Opiate agents also influence striatally induced bodily asymmetry and rotational behavior. Both MET and LEU-ENK, when injected into the GP, dose-dependently block contralateral head turning induced by electrical stimulation

[1]Present address and where requests for reprints should be addressed, University of Kentucky, Sanders-Brown Research Center on Aging, 101 Sanders-Brown Building, Lexington, KY 40536-0230.

of the CDp [50]. Naloxone has been shown to enhance apomorphine-induced rotational behavior in rats with unilateral lesions of the nigrostriatal pathway [37]. Furthermore, both acute morphine treatments and withdrawal from chronic morphine treatments cause a significant increase in striatal DA asymmetry and rotations to the side with greater DA content [19,29].

The physiological actions of ENKs within the CDp and GP along with the influence of opiate agents on motor behavior implicate a role for basal ganglia ENKs in rotational behavior and bodily asymmetry.

The first aim of the present experiment was to investigate the effects of unilateral injections of LEU and MET-ENK, as well as the opiate antagonists naloxone and naltrexone, into the CDp and GP on rotational behavior and bodily asymmetry. The second aim of this study was to investigate the effects of similar injections into sites adjacent to the CDp and GP. The adjacent sites of interest to us were nucleus accumbens, the region bordering the bed nucleus of stria terminalis and the bed nucleus of anterior commissure and the cortex, sites which are rich in ENKs and their receptors [20, 51, 53]. Since ENKs cause hyperpolarization of both CDp and GP neurons and seem to interfere with DA transmission within the CDp, unilateral injections of D-ALA-2-LEU-ENK and D-ALA-2-MET-ENK into the CDp and GP were expected to cause ipsiversive rotations and bodily asymmetry while injections of the opioid antagonists naloxone and naltrexone were expected either to cause no asymmetry or to cause contraversive rotations and asymmetry.

METHOD

Subjects

One hundred and twenty-five male Sprague Dawley rats weighing between 300–400 grams were used in this experiment.

Apparatus

Cannulae were constructed from 23 gauge stainless steel tubing with 30 gauge tubing used as a plug. The length of each cannula was 12 mm. For injection, a piece of the 30 gauge tubing 14 mm in length was connected to a 10 μl Hamilton syringe with polyethylene tubing with an i.d. of 0.28 mm. The rotometer consisted of a stainless steel bowl with a top diameter of 55 cm, bottom diameter of 15 cm and a height of 30 cm.

Surgery

The animals were anesthetized with IP injections of sodium pentobarbital (60 mg/kg) and given 0.2 ml of atropine sulphate (0.5 mg/ml) intramuscularly to help combat congestion during surgery. In half of the animals, guide cannulae were chronically implanted on the right side while in the other half they were implanted on the left. Guide cannulae were implanted in the CDp (anterior 0.5 mm, lateral 2.8 mm and ventral 5.3 mm), GP (anterior −0.8 mm, lateral 2.8 mm and ventral 6.7 mm), nucleus accumbens (anterior 1.2 mm, lateral 1.5 mm and ventral 7.5 mm), the region bordering the bed nucleus of anterior commissure and the bed nucleus of stria terminalis (anterior −0.8 mm, lateral 1.1 mm and ventral 6.5 mm) and cortex (anterior −0.3 mm, lateral 3.0 mm and ventral 2.0 mm). All of the stereotaxic coordinates were measured with the head level. The anterolateral coordinates were measured from bregma and the ventral-dorsal coordinates were measured from the skull [32]. All of the guide cannulae were implanted such that the tip of each cannula was 2.0 mm dorsal to the site of injection to allow the injection tubing to extend 2.0 mm beyond the cannula tip. All animals were allowed 5–7 days of recovery before any testing was carried out.

Drugs

Since the action of ENKs is very short lasting due to their rapid inactivation by brain endopeptidases [1] only the stable D-ALA forms of these molecules were used in this experiment. Based on pilot data on 40 animals, various doses of D-ALA-2-LEU-ENK acetate, D-ALA-2-MET-ENK acetate (Sigma Chemical Company, St. Louis, MO), naloxone hydrochloride and naltrexone hydrochloride (courtesy of Dr. Alice Young, Wayne State University, Detroit, MI) were injected into the CDp (MET-ENK: 0.25, 0.50, 1.0, 2.0 and 4.0 μg; LEU-ENK: 0.25, 0.50, 1.0 and 2.0 μg; naloxone: 0.25, 0.50, 1.0 and 2.0 μg; naltrexone: 0.25, 0.50, 1.0 and 2.0 μg) and the GP (MET-ENK: 0.25, 0.50, 1.0, 2.0 and 4.0 μg; LEU-ENK: 0.12, 0.25, 0.50, 1.0, 2.0 and 4.0 μg; naloxone: 0.25, 0.50, 1.0 and 2.0 μg; naltrexone: 0.25, 0.50, 1.0 and 2.0 μg). One group of animals received injections of D-ALA-2-LEU-ENK (0.50 μg) into the cortical layers immediately dorsal to the CDp and the GP; a second group received the same injections into an area bordering the bed nucleus of the stria terminalis and the bed nucleus of the anterior commissure. A third group received injections of D-ALA-2-MET-ENK (1.0 μg) into the nucleus accumbens. Additional animals received injections of D-ALA-2-LEU-ENK (0.50 μg) into the GP in combination with IP injections of various doses of naloxone (0.15, 0.30, 0.60 and 1.2 mg/kg) or saline. Normal saline was used as the carrier solution for all drugs and all drugs were adjusted for pH to match the saline pH. To prevent binding of the ENK to glass, all of the glass utensils were coated with a layer of Sigmacote (Sigma Chemical Company, St. Louis, MO).

Testing

Twenty minutes prior to injection, each animal was placed in the rotometer for five minutes, and the number of 360 degree turns in either direction was recorded. After fifteen minutes of rest, the plug of the cannula was removed and the injection tubing placed inside the guide cannula. Each animal received a 0.5 μl injection of drug over one minute, after which the injection tubing was removed and the plug replaced. Immediately after injection the animal was placed in the rotometer and the number of 360 degree turns in either direction recorded for five minutes. Each animal received a maximum of two injections, the two injections being 5–7 days apart and of the same drug but of different doses. Each dose of the drug was injected into the appropriate site (CDp or GP) in five animals. In addition, five animals received saline injections into the CDp and another five into the GP, and were tested in the same manner as drug injected animals. To see if the effects of the ENK injections are naloxone reversible, five groups of five animals each were given GP injections of 0.50 μg of D-ALA-2-LEU-ENK (the most effective dose of ENKs in inducing rotational behavior) along with IP injections of naloxone or saline.

To control for the spread of the injected drugs, the most effective dose of the ENKs that induced circling was injected into the cortex. The most effective dose of the ENK injec-

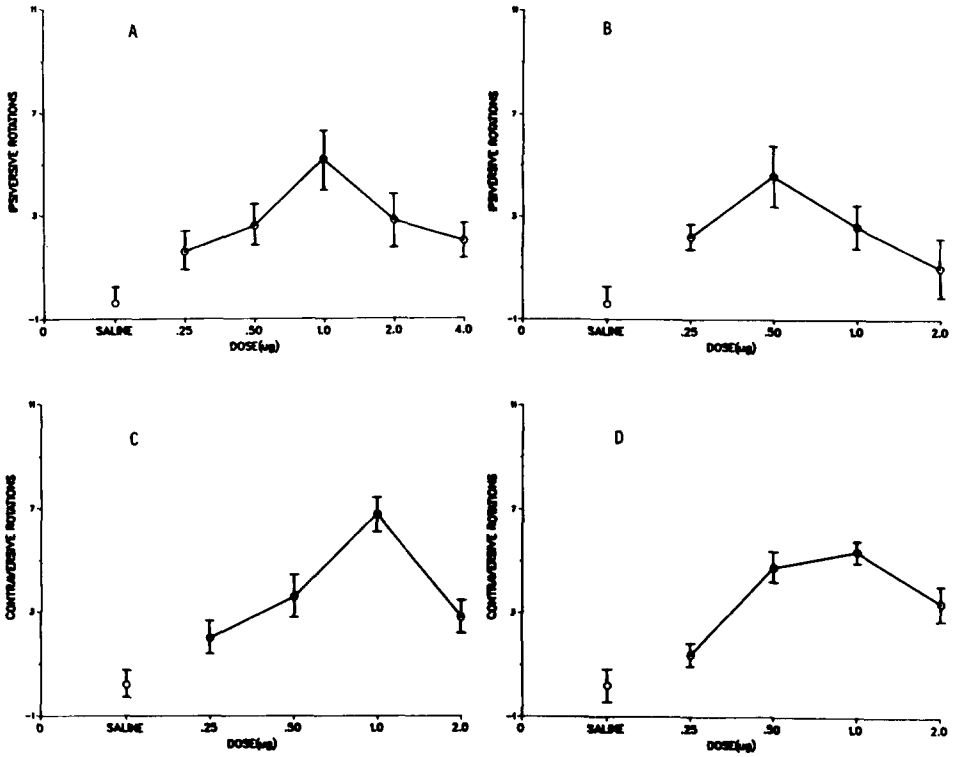

FIG. 1. Dose-response relations for the circling induced by injections of various doses of (A) D-ALA-2-MET-ENK, (B) D-ALA-2-LEU-ENK, (C) naloxone and (D) naltrexone into the CDp (turns/5 minutes). Each dose was injected into 5 animals. Data points represent means±standard errors of measurement.

tions into the CDp was injected into the nucleus accumbens and the most effective ENK dose injected into the GP was injected into the region bordering the bed nucleus of anterior commissure and the bed nucleus of the stria terminalis.

During all testing the animals were observed for degree of head tilt and bodily asymmetry. As a test of asymmetry, a modified version of the classification introduced by Costall *et al.* [10] was used [16]. According to this classification, a score of zero indicates no observable asymmetry. Animals which display slight asymmetry during 60% or more of the testing period receive a score of one; animals showing slight asymmetry during the entire test period receive a score of two; and finally, animals with severe asymmetry (head overlapping tail) during the test period receive a score of three.

Histology

After the completion of testing the animals were anesthetized with an overdose of sodium pentobarbital and perfused intracardially with a 0.9% solution of saline followed by a 10% solution of formalin. The brains were removed, frozen and sectioned at 40 μ. The sections were mounted on slides and stained using a Cresyl Violet Nissl Stain. Only data from animals having the tip of the injection tubing at the correct site were used.

RESULTS

The difference between the number of pre and post injection rotations exhibited by each animal make up the circling data in this experiment. D-ALA-2-MET-ENK, D-ALA-2-LEU-ENK, naloxone and naltrexone produced dose-dependent increases in circling when injected into the CDp and the GP (Figs. 1–2). These increases in rotational behavior were in the predicted direction for each drug. Analyses of variance performed on the circling data yielded significant effects for all drugs injected into the CDp (MET-ENK: $F(5,24)=12.1$, $p<0.001$; LEU-ENK: $F(4,20)=5.08$, $p<0.005$; naloxone: $F(4,20)=25.3$, $p<0.001$; naltrexone: $F(4,20)=41.6$, $p<0.001$) and the GP (MET-ENK: $F(5,24)=17.7, p<0.001$; LEU-ENK: $F(6,28)=14.7, p<0.001$; naloxone: $F(4,20)=22.8, p<0.001$; naltrexone: $F(4,20)=10.9$, $p<0.001$).

Within the CDp, all doses of MET-ENK injected caused significantly higher numbers of ipsiversive rotations than saline injections and 1.0 μg of this drug resulted in a significantly higher number of ipsiversive rotations than all other doses (Newman-Keuls, $p<0.05$). Of the doses of LEU-ENK injected into the CDp, only 0.50 and 1.0 μg caused significantly higher ipsiversive circling than saline (Newman-Keuls, $p<0.05$). Naloxone, in every dose injected into the CDp, produced significantly more contraversive circling than saline and a dose of 1.0 μg resulted in a significantly higher number of rotations than the other doses used (Newman-Keuls, $p<0.05$). Finally, every dose of naltrexone injected into the CDp resulted in significantly higher numbers of contraversive rotations than saline, and 0.50 and 1.0 μg caused significantly higher numbers of rotations than the other doses used (Newman-Keuls, $p<0.05$).

Every dose of MET-ENK injected into the GP produced ipsiversive rotations which were significantly higher than that produced by saline injections, with 1.0 and 2.0 μg of MET-ENK causing significantly higher numbers of rotations

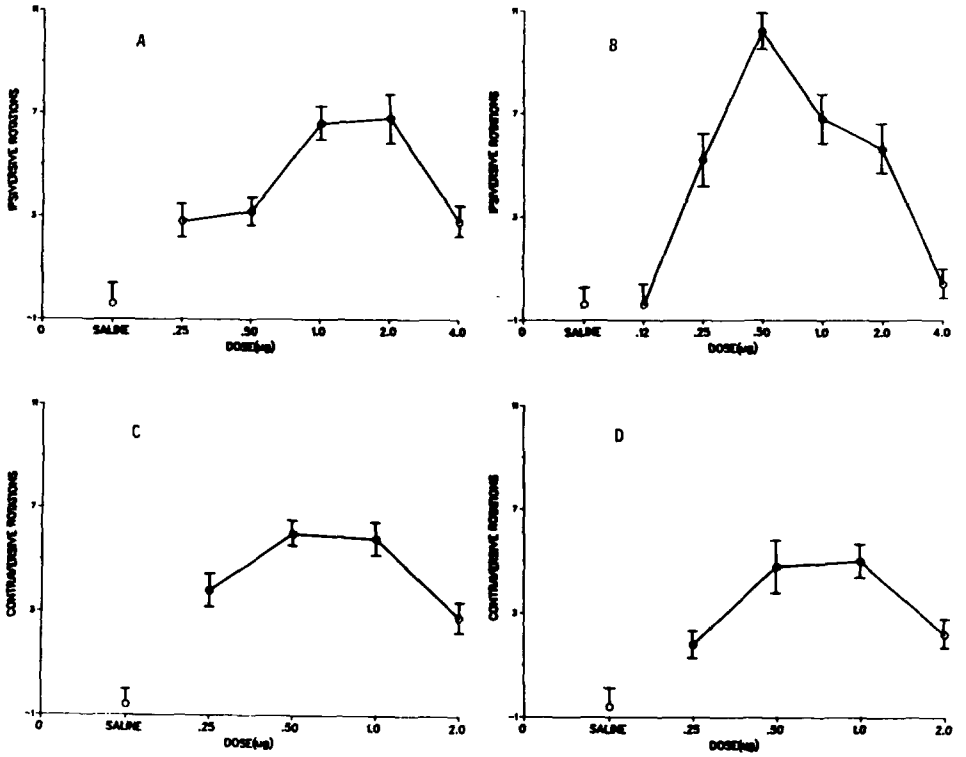

FIG. 2. Dose-response relations for the circling induced by injections of various doses of (A) D-ALA-2-MET-ENK, (B) D-ALA-2-LEU-ENK, (C) naloxone and (D) naltrexone into the GP (turns/5 minutes). Each dose was injected into 5 animals. Data points represent means±standard errors of measurement.

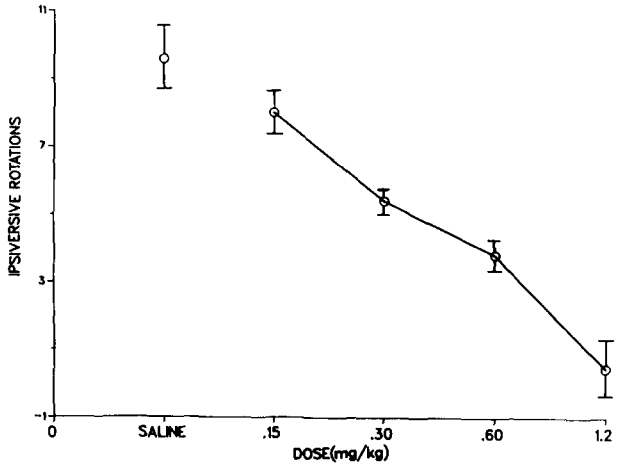

FIG. 3. Dose-response relations for the blocking effect of various doses of naloxone (IP) upon circling induced by injections of 0.50 μg of D-ALA-2-LEU-ENK into the GP (turns/5 minutes). Each dose was injected into 5 animals. The data points represent means±standard errors of measurement.

than the other doses injected (Newman-Keuls, $p<0.05$). Injections of all doses of LEU-ENK into the GP, except 0.12 and 4.0 μg, produced more ipsiversive rotations than saline, and 0.50 μg of this drug was the most effective dose of all drugs used, resulting in the highest number of rotations observed in this study (Newman-Keuls, $p<0.05$). Every dose of naloxone and naltrexone injected into the GP resulted in significantly more contraversive rotations than saline, and doses of 0.50 and 1.0 μg of these drugs caused higher numbers of contraversive rotations than other doses injected (Newman-Keuls, $p<0.05$).

Of the control injections, 0.50 μg of LEU-ENK injected into the cortex or the region bordering the bed nucleus of anterior commissure and the bed nucleus of stria terminalis failed to result in any significant circling compared to the same injections into the GP (cortex: $t=10.7$, $p<0.001$; bed nuclei: $t=9.7$, $p<0.001$). Injections of 1.0 μg of MET-ENK into the nucleus accumbens resulted in significantly fewer ipsiversive rotations than the same injection made into the CDp ($t=3.7$, $p<0.007$).

The ipsiversive rotations caused by injections of 0.50 μg of LEU-ENK into the GP (the most effective combination of ENK dose and injection site in producing rotational behavior) were dose dependently blocked by IP injections of naloxone, $F(4,20)=25.5$, $p<0.001$. All doses of naloxone except 0.15 mg/kg resulted in significantly fewer ipsiversive rotations than saline injections (Fig. 3), the most effective dose being 1.2 mg/kg which almost completely blocked ipsiversive rotations resulting from injections of 0.50 μg of LEU-ENK into the GP (Newman-Keuls, $p<0.05$).

The results of this study showed that the CDp and GP injections were followed by increased locomotion as well as asymmetry. In 90% of the cases, more rotations were recorded following injections than prior to drug treatment. That the drug treatment produced bodily asymmetry was shown by the finding that in 79% of the cases experimental

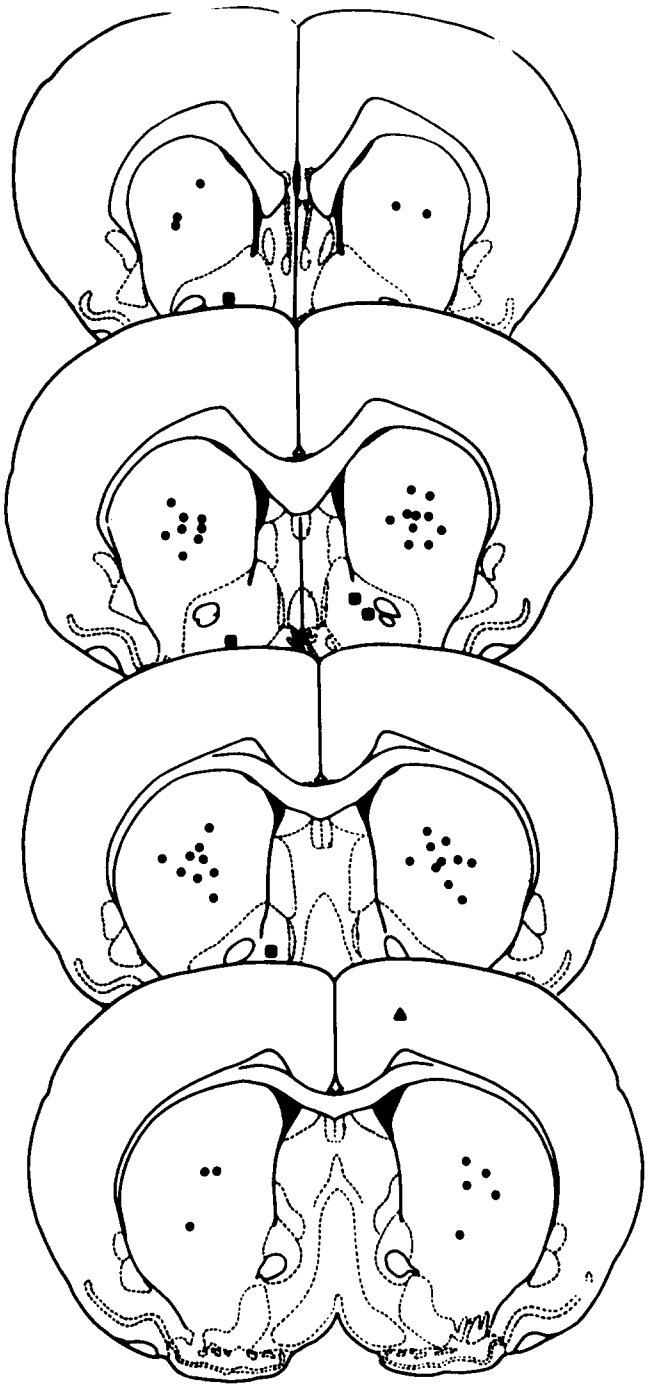

FIG. 4. Histological results showing the placements of the tip of the injection tubing in: ● CDp, ▲ cortex and ■ nucleus accumbens. Sections are, from top to bottom: 1.7 mm, 1.2 mm, 0.7 mm and 0.2 mm anterior to bregma (from Paxinos and Watson [32]).

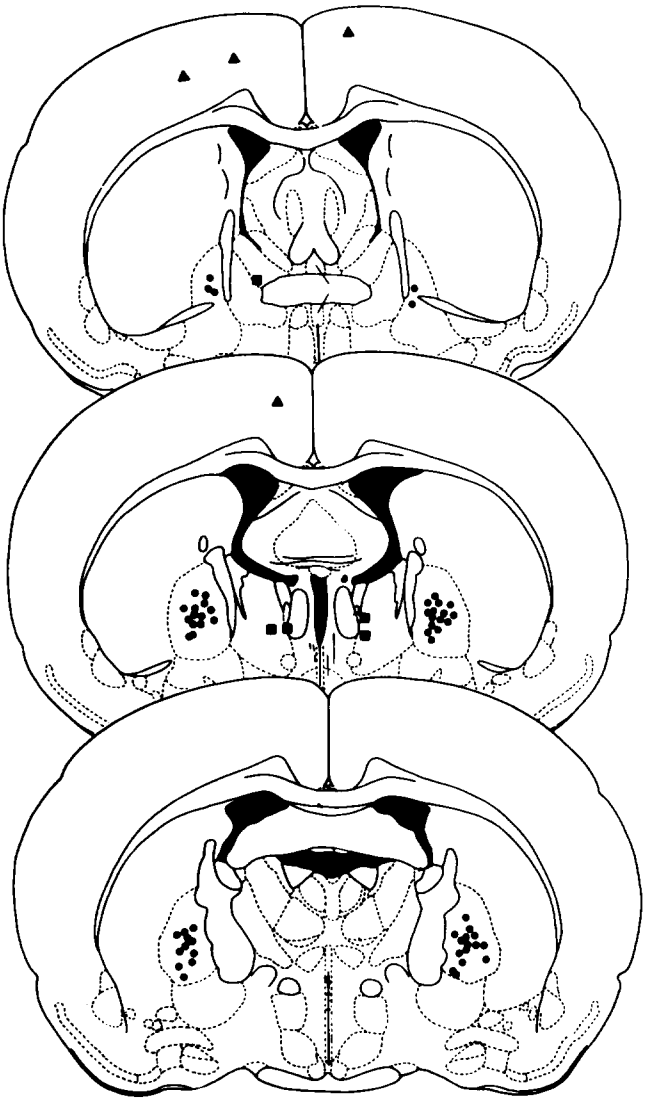

FIG. 5. Histological results showing the placements of the tip of the injection tubing in: ● GP, ▲ cortex and ■ the region bordering the bed nucleus of the stria terminalis and the bed nucleus of the anterior commissure. Sections are, from top to bottom: −0.3 mm, −0.8 mm and −1.3 mm posterior to bregma (from Paxinos and Watson [32]).

animals circled solely in the expected direction, whereas control animals circled in both directions.

The Kruskal-Wallis test applied to the asymmetry scores assigned to each subject revealed significant effects for all drugs injected into the CDp (MET-ENK: $H(5)=12.4$, $p<0.01$; LEU-ENK: $H(4)=12.2$, $p<0.01$; naloxone: $H(4)=11.2$, $p<0.01$; naltrexone: $H(4)=13.5$, $p<0.01$) and the GP (MET-ENK: $H(5)=18.5$, $p<0.01$; LEU-ENK: $H(6)=19.8$, $p<0.01$; naloxone: $H(4)=12.3$, $p<0.01$; naltrexone: $H(4)=13.8$, $p<0.01$). To determine the effects of the various doses of each drug in inducing asymmetry, a modified version of the Mann-Whitney test introduced by Ryan was used [26]. All doses of all drugs used produced significantly greater asymmetry than saline ($p<0.05$). However, no significant differences were found among the asymmetry scores resulting from the injections of various doses of each drug. The GP injections resulted in more severe asymmetries (20% more cases received a score of three) than CDp injections.

The data obtained from animals for which histology showed the tip of cannulae to have been at the incorrect site were discarded and replaced with data from additional animals with correct cannulae placements (Figs. 4–5).

DISCUSSION

The results of this experiment support the hypothesis that CDp and GP ENKs are implicated in circling and bodily asymmetry and strengthen that hypothesis by showing that rotational behavior is influenced by ENKergic substances in a dose-dependent manner.

Relatively low levels of circling were observed following CDp and GP injections of ENKergic agents when compared with the circling levels reported following other anatomical and pharmacological manipulations of these structures [35,49]. Although our injections resulted in relatively low levels of circling, our measures of asymmetry were consistently high. Thus, our findings suggest that CDp and GP ENKs play a more important role in motor asymmetry than in locomotion.

The ipsiversive rotations observed after injections of LEU-ENK into the GP appear to be a result of direct interaction of ENKs with their receptors since IP injections of naloxone dose-dependently blocked such circling. Since naloxone blocks the hyperpolarizing effect of ENK on neurons within the CDp and the GP [31,33], it is reasonable to suggest that IP injections of naloxone will block the circling observed in this experiment following injections of MET-ENK into the GP and injections of both MET and LEU-ENK into the CDp.

The physiological actions of ENKergic agents within the CDp which give rise to circling and bodily asymmetry are unclear. Behavioral studies suggest that CDp ENKs control motor behavior by inhibiting striatal DA activity [19, 29, 30, 37]. Physiological studies, however, provide little support for such a mechanism [1, 6, 7, 28]. The only possible support for such a hypothesis comes from the finding that opioid agents inhibit the adenylate cyclase induced phosphorylation of membrane proteins [8,25]. Whatever the interactions between ENKs and striatal DA prove to be, it is reasonable to suggest that CDp ENKs cause rotational behavior and bodily asymmetry by inhibiting striatal neuronal activity [15, 23, 33].

Neuronal inhibition also seems to be the mechanism through which injections of ENKs into the GP cause ipsiversive rotational behavior and bodily asymmetry [24, 31, 39, 45], since other unilateral anatomical and pharmacological manipulations of the GP which cause neuronal inhibition also result in these behaviors [35]. The contribution of the ENKergic striopallidal projection [3, 4, 9, 11, 12, 14, 27, 44] to the bodily asymmetry and rotational behavior observed in this experiment is unclear since GP injections of ENKs has been shown to have no influence on the rotational behavior induced by apomorphine in striatally lesioned rats [41].

The results of this experiment clearly indicate that basal ganglia ENKs influence circling behavior and bodily asymmetry. Therefore, any theory concerned with the mode of control that basal ganglia structures exert over motor asymmetry must take into account the actions of ENKs within these structures.

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