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Non-Dopaminergic Mechanisms in the Turning Behavior Evoked by Intranigral Opiates

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The turning effects of the unilateral intranigral injection of morphine and of different analogs of dynorphin and enkephalin were studied. All injections were made in awake rats through cronically implanted guide cannulae. Dynorphin$_{1-13}$ at a dose of 10 μg (0.6 nmol) and dynorphin$_{1-17}$ at a dose of 2 μg (0.9 nmol) produced contralateral circling when injected unilaterally in the substantia nigra (SN), lasting for about 1 h. D-Ala-dynorphin$_{1-17}$, a more stable analog of dynorphin, produced at a dose of 2 μg (0.9 nmol), a longer-lasting effect. Injections of different enkephalin analogs were also made into the SN, [D-Ser^2]-Leu-enkephalin (10 μg, 14.5 nmol) and [D-Ala^2,D-Ala^3]-Met-enkephalin (10 μg, 15 nmol) also produced contralateral circling after unilateral intranigral injection. This behavior lasted for 60–90 min, depending on the different enkephalins used. As already reported by Iwamoto and Way[18] morphine also produced contralateral circling when injected into the SN. The circling evoked by all these opiates was completely antagonized by 5 mg/kg of naltrexone s.c. In order to study the role of the dopaminergic nigrostriatal system, we made unilateral lesions of the medial forebrain bundle (MFB) with 6-hydroxydopamine (6-OHDA) and kainic acid lesions of the striatum and we looked at the effect elicited by these lesions on the behavior produced by the above compounds when injected into the SN. The lesion of the dopaminergic nigrostriatal system failed to affect either the number of turns or the duration of the contralateral circling produced by unilateral injections of morphine, dynorphin and enkephalin analogs into the SN correspondent to the lesioned side. On the other hand kainate lesions of the body of the caudate potentiated the turning induced by intra-SN morphine and dynorphin. Therefore it appears that the dopaminergic nigrostriatal system is not essential in the expression of the contraversive turning behavior produced by intranigral injections of endogenous opiates or morphine and that opiates might produce dopamine-like effects indirectly, through the inhibition of nigral non-dopaminergic output neurons.

INTRODUCTION

Anatomical, electrophysiological neurochemical and behavioral evidence accumulated in recent years has led to the concept that the substantia nigra pars reticulata plays a fundamental role in the output of the impulses originating in the striatum and in the expression of the motor and behavioral syndromes elicited by the administration of drugs which either stimulate or block striatal dopamine (DA) receptors[5,21,24]. According to this hypothesis, stimulation of striatal DA receptors results, by an intrinsic striatal mechanism, in stimulation of the activity of GABAergic striatonigral neurons projecting directly onto substantia nigra pars reticulata neurons, which are themselves GABAergic and project to the superior colliculus, dorsal mesencephalic reticular formation (MRF) and ventral thalamus[6–10]. Inhibition of nigral pars reticulata neurons thus results in disinhibition of the target areas of nigral projection. A working hypothesis, which integrates the histochemical data obained by Graybiel et al.[13], the neuroanatomical data obtained with tracing techniques[14] and the behavioral results obtained by localized lesions and intracerebral injections of receptor-specific drugs[5], envisages the existence of separate channels for specific behavioral components of the drug-induced striatal syndromes. These channels, which are supposed to originate in the mosaic-like domains of the striatum in register with specific cortical areas, would separately converge in the substantia nigra and through different nigral output neurons would finally segregate into the different targets of nigral projections[5].

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Opiate peptides are widely distributed in the basal ganglia, being particularly concentrated in the substantia nigra and in the two subdivisions of the globus pallidus[2,4,19,30,31].

The two major families of central opioid peptides, i.e. those originating from pro-enkephalin (Met-, Leu-enkephalin) as well as those originating from pro-dynorphin (dynorphin$_{1-8}$, dynorphin$_{1-17}$ etc.) are both present in the basal ganglia but show a quite separate distribution: the enkephalins being highly concentrated in the globus pallidus (lateral pallidal segment)[4,19] while dynorphins are particularly concentrated in the substantia nigra and in the entopeduncular nucleus (medial pallidal segment)[2,30,31]. This segregation acquires a particular importance if one considers that the functional significance of the substantia nigra is similar to that of the entopeduncular nucleus, since both appear to be output areas for basal ganglia and in turn quite different from that of the globus pallidus external segment, which is part of a feedback circuit intrinsic to basal ganglia. On the other hand it has been recently reported that dynorphins are contained in the terminals of a long striatonigral opiatergic pathway[31]. These results are highly suggestive of the possibility that opiate peptides convey specific functions in the basal ganglia; in particular the striatonigral dynorphinergic pathway might provide an output for striatal functions parallel to the striatonigral GABAergic pathway and eventually integrated with it. In order to investigate this possibility we studied the behavioral effects elicited by the unilateral intranigral injection of morphine and of various opiate peptides and their possible neuronal mechanism.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 280–320 g were used in all the experiments. Rats were anesthetized with Equithesin, placed on a David Kopf stereotaxic apparatus, and implanted bilaterally with stainless steel guide cannulae (22 gauge) fixed to the skull with dental cement. Guide cannulae were aimed at the substantia nigra (SN) (coordinates A-3.0, L 2.0, V 3.9 according to the atlas of Pellegrino and Cushman[26] but ended 4.0 mm dorsal to SN in order to avoid damage to this area. During the same surgical session some rats were also injected through stainless steel cannulae (28 gauge) with 6-hydroxydopamine (6-OHDA) unilaterally in the medial forebrain bundle (MFB) (coordinates A 2.2, L 1.5, V 7.9 (ref. 26)) in order to lesion the dopaminergic nigrostriatal system. The dose of 6-OHDA used was 8 μg in 4 μl of saline with 0.05% of ascorbic acid. From results consistently obtained in our laboratory and from the results of others[15,16,29,32], rats showing contralateral turning to apomorphine after unilateral 6-OHDA lesions have a higher than 90% reduction of DA concentration of the lesioned side. Therefore, a positive apomorphine test was used as a means for selecting the best lesioned rats to be injected intranigrally with opiates. Thus 7 days after surgery the rats were tested with a dose of 0.05 mg/kg s.c. of apomorphine and only the rats showing score 4 contralateral turning according to Costall et al.[3] and high speed circling (15 rpm or more) were injected intranigrally with the various opiates 7–10 days later. Similarly, some rats were lesioned with kainic acid (KA) in the striatum and implanted with nigral guide cannulae. KA lesions were performed in the caudate head (1 μg of KA in 1 μl of saline, coordinates A 2.4, L 2.8, V 5.0) or in the body (1 μg of KA in 1 μl of saline, coordinates A 1.6, L 3.0, V 5.0) and tail (0.5 μg of KA in 0.5 μl, coordinates A 0, L 4.5, V 5.0) (ref. 23). Rats with KA lesions of the caudate head received a single unilateral injection of KA while the group with KA lesions of the caudate body–tail received two unilateral injections of KA.

KA was injected at a speed of 1 μl/3 min. Sham-lesioned rats were injected with saline in the caudate head. KA and sham-lesioned rats were injected intranigrally with opiates 10–15 days after the lesions. Intranigral injections were made by replacing the stylets of the guide cannulae with a stainless steel injector protruding 4 mm from the guide cannula tip.

All the compounds were dissolved in saline and administered in a volume of 0.5 μl at a rate of 1 μl/3 min by a mechanically driven pump. Control rats were injected with saline using the same procedure. After the injections the rats were placed in plexiglas cylinders (diameter 35 cm) for behavioral observation. Circling behaviour was measured by an observer not aware of the treatments given, by counting the number of turns made every 10 or 20 min. The degree of turning (postural asymmetry) was scored according to previously reported methods[3]. After completion of

the experiment the animals were perfused with formalin, the brain included in paraffin and stained with cresyl violet, in order to ascertain the locus of the nigral injection site. 6-OHDA-lesioned rats were not perfused and the telencephalon was dissected from the mesencephalon in order to assay DA in the striatum of each side using high pressure liquid chromatography with electrochemical detection[17] and to verify by cresyl violet staining the site of intranigral injections. All peptide opiate compounds were purchased from Peninsula Laboratories, CA while 6-OHDA was purchased from Sigma.

Means and S.E.M. were calculated and the significance of the differences between means was evaluated by the Student's t-test with $P < 0.05$ for one-tail test as statistically significant.

RESULTS

Turning behavior after unilateral intranigral injection of morphine

As shown in Fig. 1, intranigral injection of 5 μg (13 nmol) of morphine produces contralateral turning

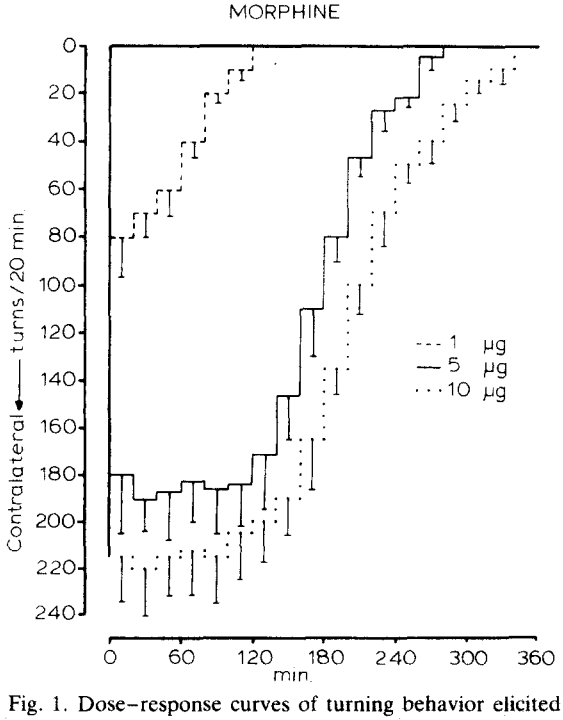

Fig. 1. Dose–response curves of turning behavior elicited by unilateral intranigral injections of different doses of morphine. In the ordinate the mean number of turns ± S.E.M. per 20 min obtained in 6 rats is indicated as a function of the time after injection.

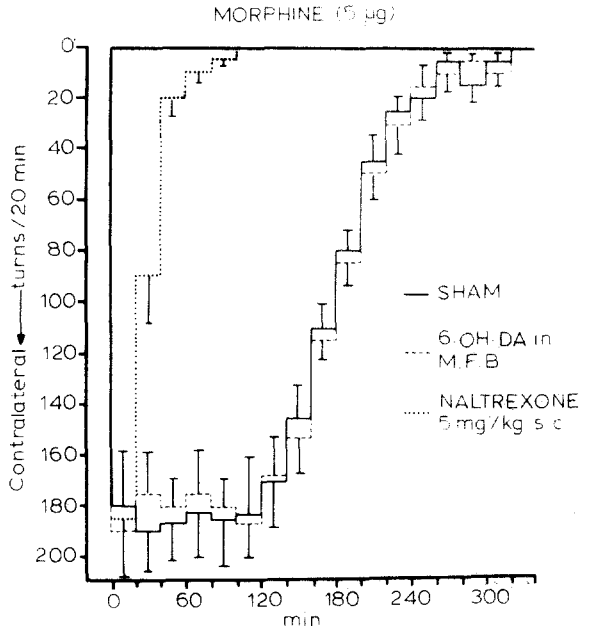

Fig. 2. Effect of 6-OHDA lesions of MFB on turning behavior elicited by morphine injected into the SN correspondent to the 6-OHDA-lesioned side. Naltrexone 5 mg/kg s.c. was administered 20 min after the intranigral injections. In the abscissa are indicated the minutes after the morphine injection and in the ordinate the mean of contralateral turns ± S.E.M. made in 20 min by 18 rats. Turning intensity of control group not significantly different from 6-OHDA-lesioned group; turning intensity of naltrexone-treated rats significantly different ($P < 0.001$) from control group.

with a tight 'head to tail' posture, starting immediately after the injection. This turning remains at a mean rate of about 4–5 rpm for about 2 h. Thereafter the number of turns starts decreasing and about 4 h later active turning behavior ceases. This turning was dose-dependent. Threshold doses for producing contralateral turning were around 1 μg (2.6 nmol). Maximal doses were around 10 μg; in fact increasing the dose, rather than producing a tighter posture, prolonged circling behavior. We obtained the most intense turning when the nigral injections were placed at level A 3 (ref. 26). On the contrary when the injection site was at level A 2.2 or 3.6 (ref. 26) the onset of the turning was delayed and the duration was shorter. Administration of 5 mg/kg of naltrexone s.c. abolished both the active turning and the postural asymmetry produced by the intranigral injection of morphine.

In order to clarify the relationship between morphine-induced contralateral turning and the dopa-

minergic nigrostriatal system, lesions of the MFB with 6-OHDA were made unilaterally and the turning behavior produced by the intranigral injection of morphine on the same side of 6-OHDA lesion was compared to that obtained in sham-operated rats. As shown in Fig. 2 morphine (5 μg) injected in the SN of the side lesioned with 6-OHDA evoked turning behavior not significantly different from that elicited from the SN of sham-operated rats. As shown in Fig. 3 also the intensity of the contralateral posture was not affected by this lesion. In these rats DA levels (μg/g) in the striatum of each side were as follows: control side, 7.5 ± 0.8; 6-OHDA side, 0.4 ± 0.1; n = 18, $P < 0.001$.

Turning behavior after unilateral intranigral injection of various enkephalins

As shown in Fig. 4 two enkephalin analogs, [D-Ser^2]-Leu-enkephalin and [D-Ala^2, D-Ala^3]-Met-enkephalin, at a dose of 10 μg (14.5 and 15 nmol, respectively) elicited contralateral turning when injected unilaterally into the SN. The intensity of circling (rpm) made by the rats was higher after [D-Ser^2]-Leu-enkephalin than after the same doses of [D-Ala^2, D-Ala^3]-Met-enkephalin. As shown in the same figure this turning was dose-dependent, and the latency was 4–5 min, while the duration was of about

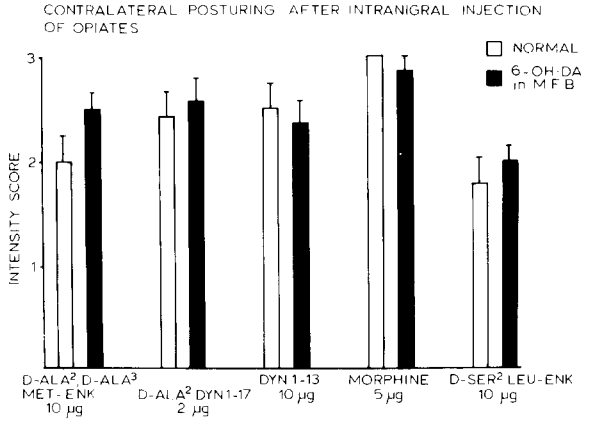

Fig. 3. Effect of morphine and different analogs of enkephalins and dynorphins injected unilaterally into the SN on the postural component of turning behavior. Results are the mean score ± S.E.M. of 5–8 rats. Empty columns represent the sham-operated rats, while the filled columns represent the 6-OHDA-lesioned rats. The score for posture was assigned according to Costall et al.[3]. The intensity of the postural asymmetry of the controls was not significantly different from that of the rats lesioned with 6-OHDA.

1 h for both [D-Ala^2, D-Ala^3]-Met-enkephalin and [D-Ser^2]-Leu-enkephalin. These compounds were capable of producing contralateral turning in 100% of the animals when injected at a dose of 20 μg at level A 3 (ref. 26). On the contrary if the injection site was at level A 2.2 or 3.6 (ref. 26) the turning was short-lasting (10–15 min) and its appearance was delayed. The administration of 5 mg/kg of naltrexone s.c. 10 min after the intranigral injection of the two enkephalins, completely abolished their effect on turning behavior. The effect of 6-OHDA lesions of the dopaminergic nigrostriatal system on turning behavior elicited by [D-Ala^2, D-Ala^3]-Met-enkephalin and [D-Ser^2]-Leu-enkephalin is shown in Fig. 5. Just as for morphine-induced turning, 6-OHDA lesions failed to reduce the number of turns or the degree of postural asymmetry produced by the two compounds injected into the SN of the side corresponding to the 6-OHDA lesion as compared to the effects elicited in sham-operated rats. In these rats DA levels (μg/g) in the striatum of each side were as follows: control side, 8.3 ± 1.1; 6-OHDA side, 0.5 ± 0.1; n = 20; $P <$ 0.001.

Turning behavior after unilateral intranigral injection of various dynorphins

As shown in Fig. 6 the unilateral intranigral injection of dynorphin$_{1-13}$ (10 μg, 0.6 nmol), dynorphin$_{1-17}$ (2 μg, 0.9 nmol) and [D-Ala^2]-dynorphin$_{1-17}$ (2 μg, 0.9 nmol) produced a contralateral turning lasting for 50–80 min, depending on the different compound used. [D-Ala^2]-Dynorphin$_{1-17}$ (2 μg), a more stable analog of dynorphin$_{1-17}$, elicited turning behavior of higher intensity and longer duration than that elicited by the less stable analog; the latency of the contralateral turning was 1–2 min after correctly placed injections, namely at level A 3 (Fig. 7). On the contrary if the injection was made at level A 2.2 or 3.6 the appearance of turning was delayed and the duration was of about 15–20 min.

As shown in Fig. 8A for the [D-Ala^2]-dynorphin$_{1-17}$, this turning was dose-dependent. The administration of 5 mg/kg of naltrexone s.c. completely abolished the turning produced by all three dynorphins. The ability of 6-OHDA lesion to interfere with the contralateral turning produced by the intranigral injection of [D-Ala^2]-dynorphin$_{1-17}$ was tested. As shown in Fig. 8B, after lesions of the dopaminergic

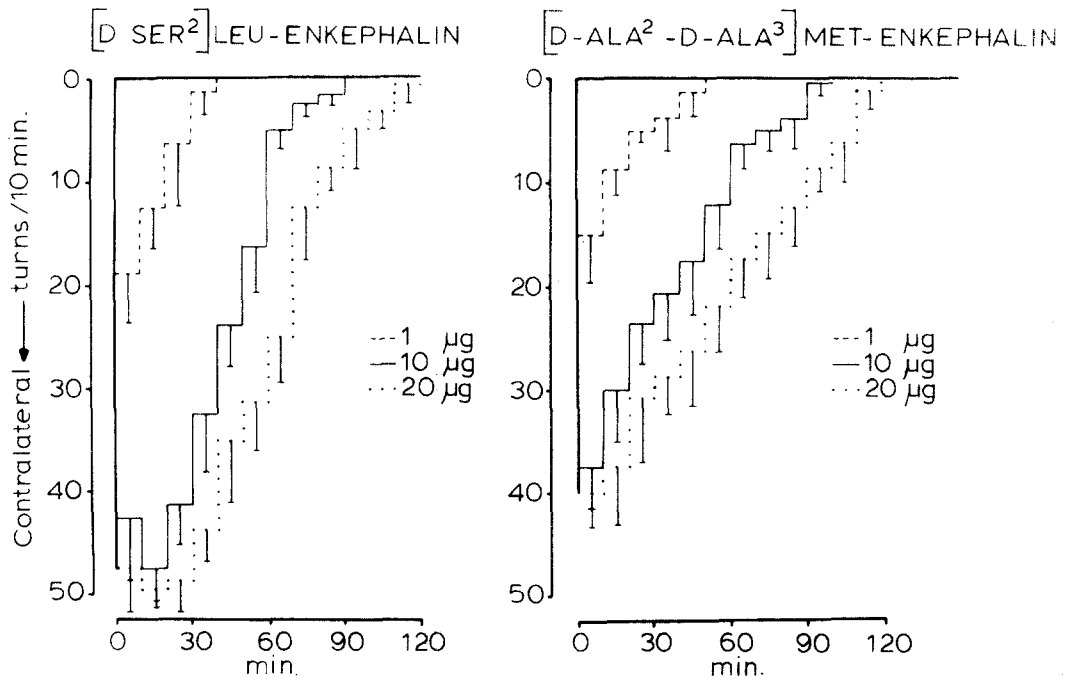

Fig. 4. Dose–response curves of turning behavior elicited by unilateral intranigral injection of 3 different doses of [D-Ser²]-Leu-enkephalin and [D-Ala², D-Ala³]-Met-enkephalin. In the ordinate the mean number ± S.E.M. of contralateral turns/10 min obtained in 7 rats is indicated as a function of the time after injection.

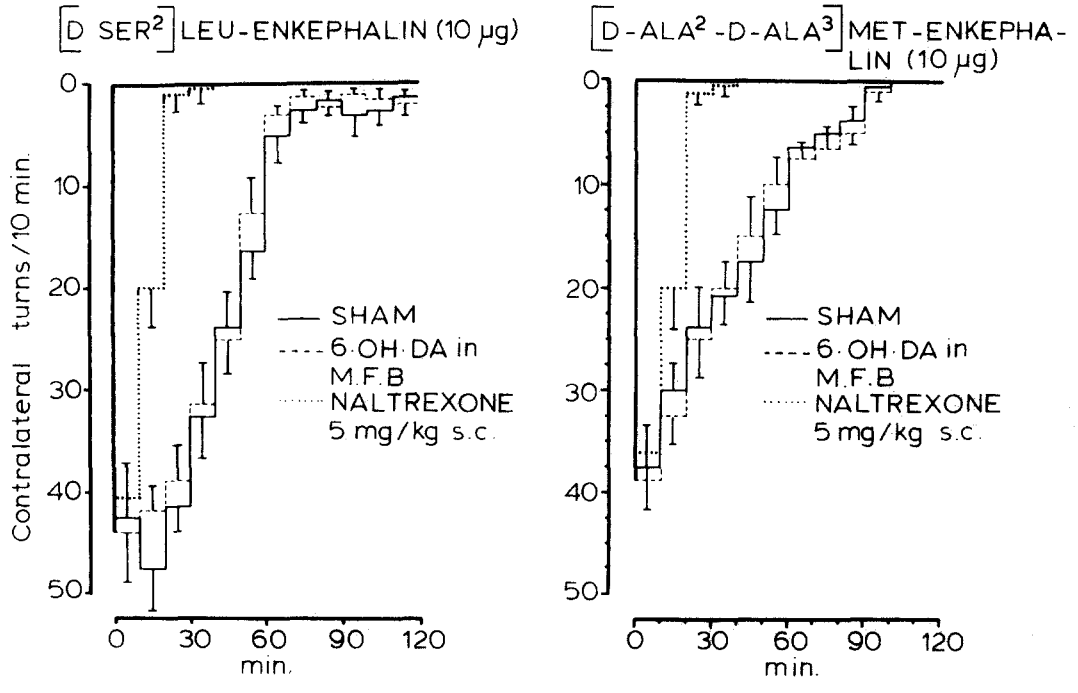

Fig. 5. Effect of 6-OHDA lesion of MFB on turning behavior elicited by intranigral [D-Ala², D-Ala³]-Met-enkephalin and [D-Ser²]-Leu-enkephalin. Naltrexone (5 mg/kg s.c.) was administered 10 min after the intranigral injection. In the ordinate the mean number ± S.E.M. of contralateral turns/10 min obtained in 11 rats is indicated as a function of the time after injection completion. Turning intensity of control group not significantly different from 6-OHDA-lesioned group; turning intensity of naltrexone-treated rats significantly different ($P < 0.001$) from control group.

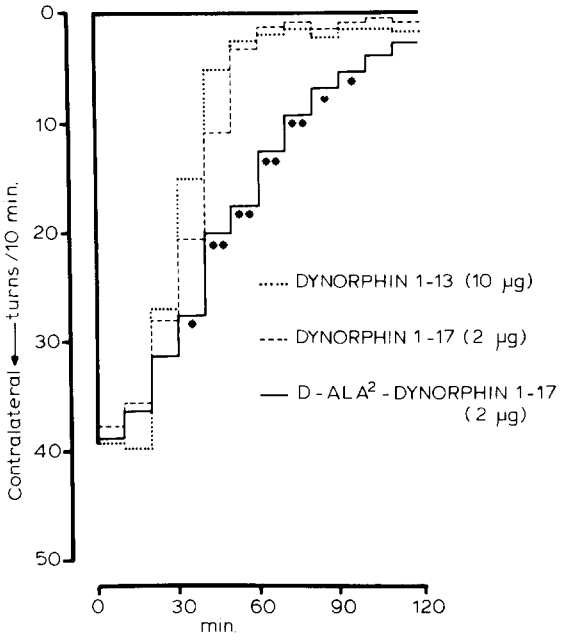

Fig. 6. Effect of dynorphin$_{1-13}$, dynorphin$_{1-17}$ and [D-Ala^2]-dynorphin$_{1-17}$ injected unilaterally into the SN. In the ordinate the mean number of contralateral turns obtained in 6 rats is reported as a function of the time after injection completion. * $P < 0.025$; ** $P < 0.005$ in respect to dynorphin 1–13 and 1–17.

nigrostriatal system no significant differences were obtained in the number of turns and in the duration of the turning produced by intranigral injection of [D-Ala^2]-dynorphin$_{1-17}$ made on the same side of the lesion when compared to sham-operated rats. As shown in Fig. 3 also, the intensity of the contralateral posture was not affected by this lesion. In these rats the DA levels (μg/g) in the striatum of each side were the following: control side, 7.8 ± 0.4; 6-OHDA side, 0.4 ± 0.1; n = 18; $P < 0.001$.

Potentiation of morphine and dynorphin induced turning by kainate lesions of the striatum

In order to investigate the role of the striatum in the turning elicited by intranigral opiates, kainate lesions were made in the body or in the head of the striatum of one side and the turning behavior obtained was compared to that obtained in sham-lesioned rats. As shown in Table I morphine produced contraversive turning in rats lesioned in the body of the striatum already at doses of 0.5 μg, which are ineffective in normal or sham-operated rats. Moreover, the turning produced by 1.0 and 2.0 μg of morphine in kainate-lesioned rats was of higher intensity than that obtained in sham-lesioned rats. In contrast, morphine appeared similarly potent and effective in rats lesioned in the head of the striatum as compared to the sham-operated ones. Also [D-Ala^2]-dynorphin$_{1-17}$ appeared more potent in producing contraversive turning when injected in the nigra of rats lesioned with kainate in the body of the striatum. Thus doses of 0.25 μg of [D-Ala^2]-dynorphin$_{1-17}$ which are ineffective in sham-lesioned rats were already active in eliciting contraversive turning in kainate-lesioned rats. Moreover, doses of 0.5 and 1.0 μg of [D-Ala^2]-dynorphin$_{1-17}$ produced turning of higher intensity when injected in the nigra of kainate-lesioned as compared to sham-lesioned rats. No such potentiation was observed if kainate lesions were placed more cranially in the striatum at the level of its head

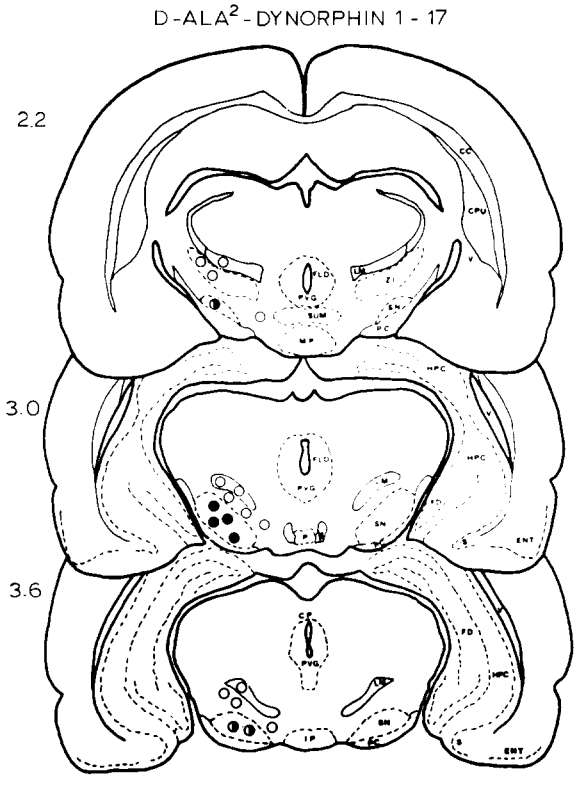

Fig. 7. Topography of the sites sensitive (filled circles) and insensitive (empty circles) to the turning elicited by intracerebral injections of [D-Ala^2]-dynorphin$_{1-17}$ (2 μg). If the injections were placed at the level marked by the half filled circle, the arising of turning was delayed and the duration shorter. SN, substantia nigra; LM, medial lemniscus; IP, interpeduncular nucleus; ZI, zona incerta.

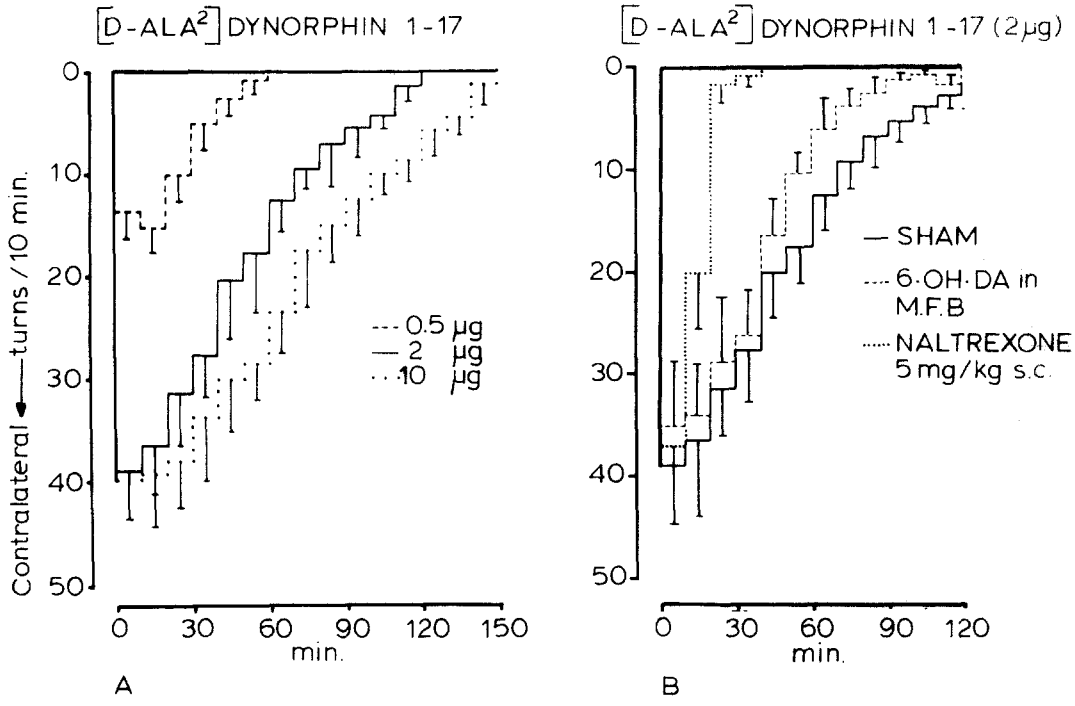

Fig. 8. A: dose–response curves of turning behavior produced by unilateral intranigral injection of 3 different doses of [D-Ala²]-dynorphin$_{1-17}$. In the ordinate the mean number ± S.E.M. of contralateral turns/10 min obtained in 6 rats is reported as a function of the time after injection completion. B: effect of 6-OHDA lesion of MFB on turning behavior elicited by intranigral [D-Ala²]-dynorphin$_{1-17}$. Naltrexone 5 mg/kg s.c. was administered 10 min after the intranigral injection. In the ordinate the mean number ± S.E.M. of the contralateral turns/10 min obtained in 9 rats is reported as a function of the time after injection completion. Turning intensity of control group not significantly different from 6-OHDA-lesioned group; turning intensity of naltrexone-treated rats significantly different ($P < 0.001$) from that of control group.

DISCUSSION

In this study we have described the ability of various opiates such as morphine, enkephalins and dynorphins to elicit contraversive turning when injected unilaterally in the nigra of the rat. Since this behavior is abolished by the peripheral administration of naltrexone, a potent opiate antagonist, it appears that the substantia nigra contains specific opiate receptors capable of influencing the activity of nigral neurons to a degree sufficient for eliciting a motor response.

Contraversive turning behavior elicited from the nigra could be due, in principle, to stimulation of nigrostriatal DA neurons or to inhibition of GABAergic pars reticulata neurons. Previous studies by Iwa-

TABLE I

Effect of striatal kainic-acid lesion on the contralateral turning produced by intranigral opiates

Active contralateral turning behavior (mean ± S.E.M. turns) was measured for its whole duration after intranigral injection of the various opiates in the three groups of rats indicated in the table. n = number of rats in each group.

Opiate (Dose, μg)	*Sham (n = 5)*	*KA-lesioned (head) (n = 9)*	*KA-lesioned (body) (n = 10)*
Morphine (0.5)	100 ± 22	170 ± 39 n.s.	580 ± 181**
Morphine (1)	350 ± 70	600 ± 110 n.s.	1030 ± 400**
Morphine (2)	1100 ± 230	1700 ± 350 n.s.	3110 ± 780**
[D-Ala²]-Dynorphin$_{1-17}$ (0.25)	15 ± 4	31 ± 10 n.s.	80 ± 20**
[D-Ala²]-Dynorphin$_{1-17}$ (0.5)	51 ± 12	92 ± 21 n.s.	260 ± 78**
[D-Ala²]-Dynorphin$_{1-17}$ (1)	150 ± 30	205 ± 41 n.s.	503 ± 110**

** $P < 0.001$; n.s., not significantly different from sham-operated rats.

moto and Way[18] attributed the contraversive turning elicited by intranigral opiates such as morphine and other narcotic analgesics as well as β-endorphin, to stimulation of dopaminergic nigrostriatal neurons on the basis of the observation that 6-OHDA lesions or systemic haloperidol prevented the contraversive turning response. In contrast with the results of Iwamoto and Way[18] we have found that 6-OHDA lesion leaves unchanged the ability of opiates to elicit contraversive turning from the nigra, indicating that DA-neurons are not essential for opiate-induced turning.

In agreement with a non-dopaminergic mediation of opiate-induced contraversive turning is the finding that lesions of the striatum by kainic acid potentiate the turning instead of reducing it, as expected if turning was mediated by activation of a nigrostriatal dopaminergic system. In fact lesions of the striatum or of the striatonigral projections are known to impair a variety of behavioral and motor responses which are mediated by activation or blockade of striatal DA receptors. The potentiation of opiate-induced contraversive turning by striatal lesions appears related to a lesion of a specific subdivision of the striatum, i.e. its central portion (body) as lesions of the head were less effective in potentiating the opiate-induced turning. This area coincides with that providing the major proportion of GABAergic projections[11] to the nigra and with the distribution of enkephalin positive neurons in the striatum[13]. This might suggest that the potentiation is related either to a denervation supersensitivity of opiate receptors following lesion of an opiatergic striatonigral input or to changes in the basal level of activity of nigral neurons sensitive to opiate actions following removal of a tonic input (GABAergic; opiatergic) to the nigra. The reason for the discrepancy between our results and those of Iwamoto and Way[18] is unclear; however, certain basic differences in the intracerebral injection technique might have contributed to it. In fact, while we used animals prepared with chronically implanted guide cannulae in order to perform the intracerebral injections in awake, freely moving rats, Iwamoto and Way[18] performed their intracerebral injections in anesthetized rats mounted on a stereotaxic apparatus. As we have pointed out elsewhere[5], the acute intracerebral injection technique in anesthetized animals can result in artifacts due to interaction between the anesthesia and the effect of the drug injected intracerebrally. In the case of intranigral opiates, higher turning rates are obtained if they are injected into the nigra of anesthetized rats (Morelli and Di Chiara, unpublished observations) probably because of interaction between the postanesthetic excitatory phase and the postural asymmetry produced by intranigral opiates. In this situation, any pharmacological or physical manipulation of brain function which, like haloperidol or 6-OHDA lesions, is capable of interfering with postanesthetic excitation, will reduce turning intensity. Our results therefore suggest that contraversive turning behavior after intranigral opiates can be mediated exclusively by non-dopaminergic nigral neurons. This conclusion is strengthened also by the observation that bilateral intranigral injection of morphine (10 μg) produces a dose-related and long-lasting stereotyped behavior characterized by sniffing head-nodding, licking and gnawing, which is blocked by naltrexone (5 mg/kg s.c.) but is resistant to high doses of peripheral haloperidol (1 mg/kg s.c.) (Morelli and Di Chiara, in preparation). Thus, the turning and stereotypy induced by intranigral opiates recognizes a quite different mechanism from that elicited by injection of opiates into another dopaminergic nucleus such as the ventral tegmental area[20]. The actions of intranigral opiates have many similarities with those of GABA-mimetic compounds[28]. Thus, both compounds produce contraversive turning and stereotypy from the nigra and these effects are resistant to haloperidol and are not prevented by 6-OHDA lesion of ascending DA neurons[28,33]. The differences are in fact only quantitative, as the intensity of turning behavior elicited by opiates does not reach the high rates of circling evoked by muscimol. This difference might reflect a difference in the basic mechanism of the neuronal action of these two classes of compounds. The similarities allow for the hypothesis that, like the turning behavior elicited by intranigral muscimol, also that evoked by intranigral opiates is due to depression of GABAergic neurons of pars reticulata projecting to extra nigral areas such as the superior colliculus, MRF and ventral thalamus[9].

If we extend the action of exogenously administered agonists to that of endogenously released opiates, our results, coupled to the experimental demonstration of a long striatonigral dynorphinergic

pathway[31], suggest that striatal impulses are funnelled to the nigra through at least two separate but parallel pathways, the GABAergic and the dynorphinergic one; indeed, when injected in the nigra, dynorphins elicited turning effects superimposable to those of morphine and, from all points of view, indistinguishable from them. It should be pointed out here, that no indication of a specificity in terms of opiate receptor subtypes could be obtained. Thus [D-Ser^2]-Leu-enkephalin and [D-Ala^2, D-Ala^3]-Met-enkephalin which are δ-receptor agonists, are just as active as dynorphins which are putative κ-receptor agonists. Morphine, on a molar basis, appears only slightly less potent than the κ-agonist. The reason for this apparent lack of receptor subtype specificity might be complex. Pars reticulata neurons might possess all three receptor subtypes, alternatively nigral neurons might possess a single receptor subtype (κ) capable of recognizing to a certain extent, also μ- and δ-agonists. On the other hand the specificity of opiates in terms of receptor subtypes at central level does not appear as strict as in peripheral preparations. Thus, the concept of dynorphins as pure κ-receptor agonists has been revised in favour of a mixed μ and κ specificity[12-22]. On the other hand, using the local intracerebral administration a correlation between dose of the drug administered, affinity for the receptors and effect elicited does not hold necessarily because of the influence of additional variables, like rapid diffusion of the drug out of the area and, in the case of peptides, local metabolic degradation and difficulty in reaching the sites of action (receptors) because of poor lipid solubility and steric hindrance.

For all these reasons it would be unwise to make a firm statement regarding the kind of opiate receptor subtype of the substantia nigra which mediates the effects here described. On the contrary, this question could be answered by the use of specific antagonists for opiate receptor subtypes. Experiments in this direction are in progress.

The present study provides additional points of similarity between GABA and opiates in the basal ganglia. Thus, GABA and opiates are contained in morphologically similar neurons of the striatum (medium size spiny neurons)[1,27] and, in some of these neurons, the two transmitters actually coexist[23,25]. The present study now shows that, at least in the nigra, GABA and opiates exert qualitatively similar behavioral effects. With these premises, an appealing hypothesis would be that in the basal ganglia an essential feature of the function of the long striatal projection neurons is a coupling between the action of GABA and that of opiates. Such coupling might take the form of a facilitatory influence exerted by opiate receptor stimulation on GABA-mediated inhibition.

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