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# Interactions Between Enkephalin and Dopamine in the Control of Locomotor Activity in the Rat: A New Hypothesis

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AGMO, A. AND N. DE AVILA. *Interactions between enkephalin and dopamine in the control of locomotor activity in the rat: A new hypothesis.* PHARMACOL BIOCHEM BEHAV 22(4) 599–603, 1985.—D-ala$^2$-met$^5$-enkephalinamide (DALA) was found to be without effect on motility when injected in doses from 10 to 40 μg. When ICV injection of DALA was combined with IP injection of amphetamine, DALA markedly enhanced the stimulatory effect of amphetamine. The effects of DALA + amphetamine could be partially antagonized by naloxone. The locomotion-reducing effect of the dopamine antagonist pimozide was not affected by concurrent administration of DALA. These data suggest a complex interaction between opiates and dopamine. It is suggested that the effects of DALA are best explained assuming that the opiate inhibits GABAergic neurotransmission.

Enkephalin Amphetamine Naloxone Pimozide Locomotor activity

THE influence of opiates on locomotor activity has recently attracted considerable attention. Morphine has been shown to have a biphasic effect on motility in rats, initially causing a depression and then a stimulation [22,25]. Of the naturally occurring opiate peptides, β-endorphin has been shown to induce a state of immobility and muscular rigidity, by some authors compared to the catalepsia seen in schizophrenia [4]. The synthetic opiate agonist, d-ala$^2$-methionine enkephalinamide (DALA), has effects similar to those of morphine, in that high doses produce an initial reduction of motility, followed by a stimulation [5,15]. Direct injection of DALA into the ventral tegmental area, n. accumbens or globus pallidus have also been shown to increase motility [6, 19, 23]. Moreover, the opiate antagonist naloxone has been found to reduce locomotor activity in rats and mice [8, 18, 27]. These data might indicate that the opiates are involved in the control of motility.

There are also some studies suggesting an interaction between opiates and dopamine (DA) in the control of locomotor activity. Naloxone reduces the increase in locomotion caused by amphetamine and moderate doses of apomorphine [17], while the morphine-induced stimulation of motility is antagonized by the catecholamine synthesis inhibitor α-methyl-p-thyrosine [22]. However, there are at present no data as to the possible interaction between dopaminergic drugs and the enkephalins in relation to motility.

It was thought of interest to study the effects of DALA on locomotor activity after stimulation or inhibition of dopaminergic neurotransmission. In the present experiments, the effects of DALA alone or in combination with the DA stimulating drug amphetamine or the DA receptor antagonist pimozide were studied.

## METHOD

### *Subjects*

Male Wistar rats (350–450) g from a local colony were used in all experiments. The animals were maintained under a 12 hr light/dark cycle, and fed commercial rat pellets ad lib.

Rats to be injected with DALA were stereotaxically implanted with a 21 gauge guide cannula in the left cerebral ventricle (coordinates: 0.1 mm anterior to bregma, 1 mm lateral to the midline and 4.5 mm below the dura. The head was fixed in such a way that lambda was 1 mm lower than bregma.) under pentobarbital anesthesia (50 mg/kg) supplemented with ether when necessary. After the operation the animals were allowed to recover for at least 5 days. Correct implantation was verified by the injection of 5 μl methylene blue at the time of sacrifice. The brain was then removed, cut and examined under a dissection microscope.

### *Drugs*

D-ala$^2$-met$^5$ enkephalinamide acetate (SIGMA Chemical Co., St. Louis, MO) was dissolved in physiological saline immediately before use, and injected ICV in a volume of 5 μl during 60 sec. The tip of the 27 gauge injection cannula protruded about 0.3 mm from the guide cannula. The former was left in place for 60 sec after injection, and was then replaced by a dummy cannula. During injections the animals were restrained manually.

DL-amphetamine sulfate (benzedrine, Ministry of Health and Assistance, Mexico) and naloxone HCl (Endo, Garden City, NY and Endo de México, Mexico City) were dissolved in physiological saline and injected SC and IP respectively,

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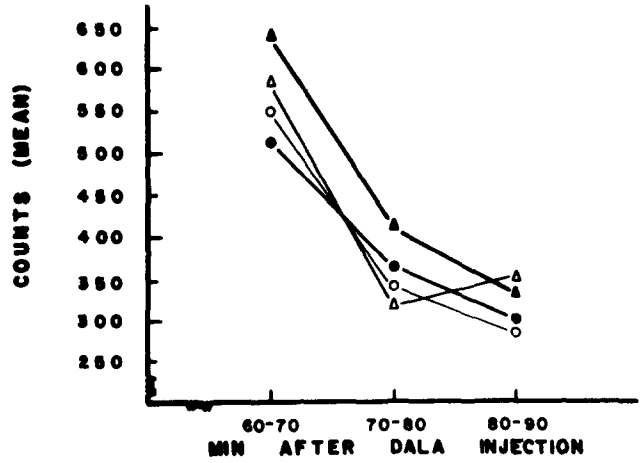


FIG. 1. Effects of DALA on locomotor activity. Open circles, physiological saline; full circles, DALA 10 μg; open triangles, DALA 20 μg; full triangles, DALA 40 μg. N=9. ANOVA revealed no differences between groups, nor any group-time interactions.

TABLE 1

LOCOMOTOR ACTIVITY AFTER TREATMENT WITH AMPHETAMINE ALONE OR AMPHETAMINE + NALOXONE

| Treatment (mg/kg) | Counts/10 min (mean ± SE) | N |
|---|---|---|
| Saline | 611 ± 78 | 14 |
| Amphetamine 0.75 | 812 ± 128† | 14 |
| Amphetamine 1.5 | 983 ± 106‡ | 14 |
| Amphetamine 3.0 | 1070 ± 121‡ | 14 |
| Amphetamine 1.5 + NaCl | 753 ± 64 | 15 |
| Amphetamine 1.5 + naloxone 0.8 | 694 ± 67 | 15 |
| Amphetamine 1.5 + naloxone 3.2 | 635 ± 66 | 15 |

The animals were observed for 10 min.
†Different from saline ($p<0.05$, DMRT).
‡Different from saline ($p<0.001$, DMRT).

in a volume of 1 ml/kg. Pimozide (Janssen, Bersee, Belgium and Janseen de México, Mexico City) was dissolved in a few drops of glacial acetic acid and then diluted with hot physiological saline to the appropriate concentration. pH was adjusted to about 5.5 with 1 M NaOH before injection. This drug was injected in a volume of 5 ml/kg.

The intervals between injection and observation were the following: DALA and pimozide, 60 min; dl-amphetamine, 40 min; naloxone, 15 min. All doses mentioned refer to the form of the compound indicated above.

*Behavioral Observation*

The animals were observed for gross behavioral changes in their home cage for 15 min after ICV injection. The following behaviors were registered: Immobility (complete absence of movement for more than 60 sec consecutively); wet-dog shakes (rapid shaking of the head, forelimbs, thorax and upper abdomen). Locomotor activity was measured using a cylindrical steel cage (diameter 24 in, height 15 in) equipped with 6 photocells covered by infrared filters, placed 2.5 cm above the grid floor. Before drug treatment, the animals were habituated to the apparatus during 3 ten min sessions. At that time, their activity had reached a stable level. During experiments, the animals were observed for a 10 or a 30 min period. In this latter case, activity was recorded every 10 min. One animal was observed at a time. All experiments were performed between the 2nd and 6th hour of the dark period.

The drugs or combination of drugs were administered according to a latin square design or in counterbalanced order when adequate, in such a way that all subjects received all the doses of a given drug or were used as their own control. The interval between drug treatments was 48 hr when DALA alone was injected, and 7 days for all other treatments. These intervals should be sufficiently long to avoid the development of tolerance or carry-over effects of previous treatments. No animal was treated with more than 1 drug or combination of drugs.

*Statistical Analysis*

An ANOVA for repeated measures followed by Duncan's Multiple Range Test (DMRT) were used for between-group comparisons in experiments with more than 2 treatments. A $t$-test was used for evaluation of experiments with 2 treatments. In this latter case, the significance levels given are for 2-tailed tests.

## RESULTS

*The Effects of DALA Alone*

The locomotor activity observed between 60 and 90 min after the ICV injection of DALA, 10, 20 or 40 μg was not significantly different from that of animals injected with saline (Fig. 1). At the highest dose, 7 of the 9 animals showed immobility during the 15 min following injection, and 5 of the animals exhibited intense wet-dog shakes. At the dose of 20 μg, 6 of the 9 animals displayed immobility and 8 of them wet-dog shakes. Of the 9 animals injected with 10 μg, one showed wet-dog shakes, but none of them showed immobility. In the saline-treated animals, no wet-dog shakes or immobility were observed. It should be noted that these behavioral effects of DALA had disappeared completely after about 30 min, and thus did not affect locomotion during the observation period.

It was decided to use a dose of 20 μg in the further experiments, since this was the lowest dose that induced clear behavioral changes.

*The Effects of DALA Combined With Amphetamine*

In Table 1, the effects of different doses of dl-amphetamine on locomotion are shown. It can be seen that a dose of 1.5 mg/kg causes an intermediate stimulation. That dose was used in all further experiments.

DALA + dl-amphetamine caused a significantly larger stimulation of locomotion than did saline + dl-amphetamine. This holds for the total activity counts 60–90 min after DALA injection, as well as for the activity 70–80 and 80–90 min postinjection. However, during the first 10 min of the observation period there was no significant difference between the treatments (Fig. 2).

An attempt was made to inhibit the effects of DALA with a low dose of naloxone. Since naloxone by itself can reduce locomotor activity and inhibit the effects of am-

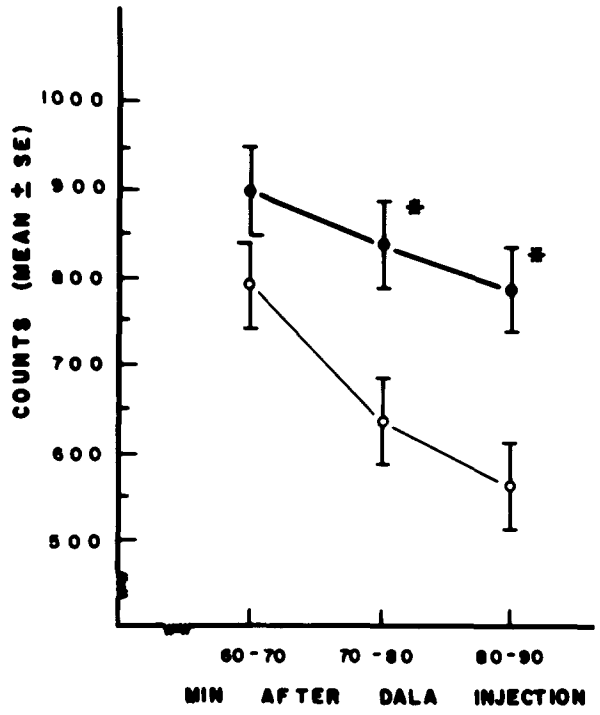


FIG. 2. Effects of DALA (20 μg) combined with amphetamine (1.5 mg/kg) on locomotor activity. Open circles, physiological saline ICV + amphetamine IP; full circles, DALA ICV + amphetamine IP. N=9. *Significantly different from physiological saline + amphetamine ($p<0.05$, $t$-test).

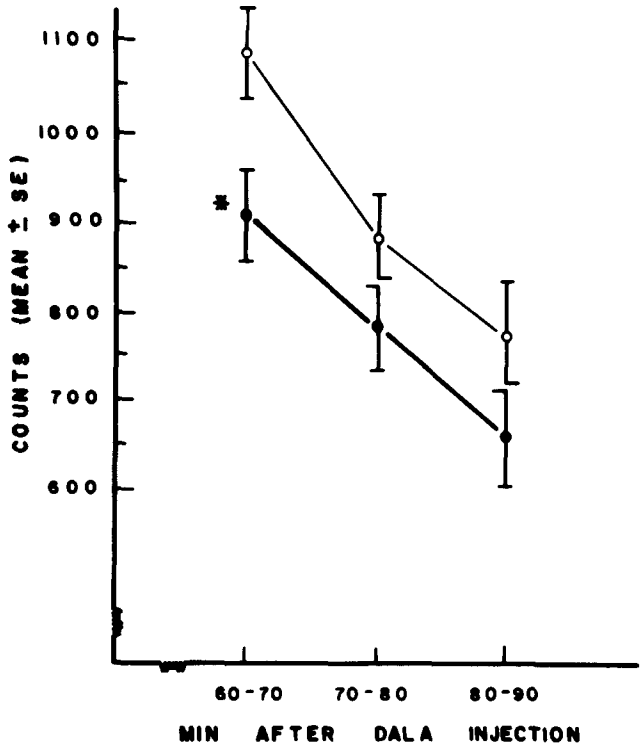


FIG. 3. Effects of DALA (20 μg) + amphetamine (1.5 mg/kg) after administration of naloxone 0.8 mg/kg. Open circles, DALA + amphetamine + physiological saline; full circles, DALA + amphetamine + naloxone. N=9. *Significantly different from DALA + amphetamine + physiological saline ($p<0.05$, $t$-test).

phetamine, the effects of naloxone after dl-amphetamine injections were investigated. From Table 1 it can be seen that a naloxone dose of 0.8 mg/kg had only a very small effect on locomotion, whereas a dose of 3.2 mg/kg produced a reduction of borderline statistical significance. In a separate experiment, 9 rats were injected with DALA + dl-amphetamine + saline or DALA + dl-amphetamine + naloxone 0.8 mg/kg. It was found that naloxone reduced the activity during the first 10 min of the observation period. Total activity counts as well as the activity 70–80 and 80–90 min postinjection were unaffected by naloxone (Fig. 3). For some reason, the animals in this experiment showed generally a higher activity than in the preceding experiment. However, this fact does not affect the conclusion, i.e., that naloxone partially antagonizes the effects of DALA.

TABLE 2

LOCOMOTOR ACTIVITY AFTER TREATMENT WITH PIMOZIDE

| Treatment (mg/kg) | Counts/10 min (mean ± SE) |
|---|---|
| Saline | 486 ± 31 |
| Pimozide 0.25 | 481 ± 37 |
| Pimozide 0.5 | 379 ± 40† |
| Pimozide 1.0 | 267 ± 29‡ |

The animals were observed for 10 min.
†Different from saline ($p<0.01$, DMRT).
‡Different from saline ($p<0.001$, DMRT).

*The Effects of DALA Combined With Pimozide*

In Table 2, the effects of different doses of pimozide on locomotion are shown. A dose of 0.5 mg/kg was considered adequate for the following experiment.

DALA 20 μg injected immediately before pimozide had no effect on motility observed 60–90 min after injection (Fig. 4).

## DISCUSSION

The time interval chosen for observation of locomotor activity (60–90 min postinjection) is the interval when the initial effects of the enkephalin (immobility and wet-dog shakes) have weaned off, and the stimulatory effect is maximal [15]. However, in contrast to earlier studies [5,15], we found no effects of DALA on locomotion during this interval when the peptide was injected into otherwise untreated animals. This might be due to differences in experimental procedure. For instance, in the other studies, experiments were performed during the light phase of the light/dark cycle. It is well known that the rat presents a circadian rythm in activity, with a minimum during the first hours of the light period and a maximum during the first hours of the dark period ([26 ] and reference therein). The enkephalin concentration in the brain apparently shows a similar circadian variation, i.e., with a minimum at the beginning of the light period and a maximum at the beginning of the dark period [2]. It is possible that exogenous enkephalin stimulates ac-

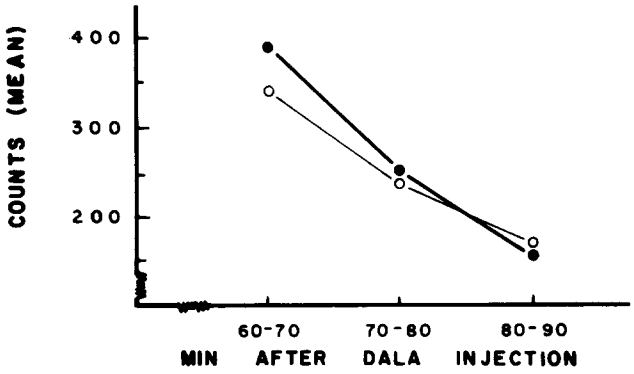


FIG. 4. Effects of DALA (20 μg) combined with pimozide (0.5 mg/kg) on locomotor activity. Open circles, physiological saline ICV + pimozide IP; full circles, DALA ICV + pimozide IP. N=9.

tivity when administered at a time when spontaneous activity as well as brain enkephalin content are minimal. In the present study, however, experiments were performed during the first hours of the dark phase, when both spontaneous activity and brain enkephalin content are maximal. The observation that the hyperalgesic effect of naloxone is maximal at the time when pain sensitivity is minimal (late afternoon) [11] is an example of time-of-administration-dependent effects of drugs acting at opiate receptors.

Several substances are known to induce wet-dog shakes in rats ([10] and references therein), among these the enkephalins. The enkephalin-induced wet-dog shakes are inhibited by naloxone, suggesting an involvement of opiate receptors in the action of this substance. For doses of enkephalin within the range 5–40 μg there is no direct dose-effect relation [9]. This is in agreement with the results obtained in our study. It was not possible, though, to determine if the wet-dog shakes could be inhibited by naloxone, since this drug was injected at a time when these had terminated spontaneously. With regard to immobility, no comparison can be made with the earlier studies, since they don't present any quantitative data.

When dopaminergic neurotransmission was stimulated by amphetamine, DALA showed a clear stimulating effect on locomotion. On the other hand, DALA did not influence the effects of the DA antagonist pimozide. These data show that DALA cannot be a DA agonist, because in that case it should have stimulated motility in the absence of amphetamine and reduced the inhibitory effects of pimozide. Neither can DALA be a DA antagonist, because then it should have reduced the effects of amphetamine and reinforced the effects of pimozide and inhibited motility on its own. There are also biochemical data showing that biologically active opiate agonists do not bind to DA receptors, except at very high concentrations [7].

It has recently been shown that the inhibitory effects of naloxone on motility can be blocked by the GABA antagonists bicuculline and picrotoxin, and it has been suggested that naloxone acts by disinhibiting GABAergic neurons [1]. If this were the case, the enkephalins would inhibit GABAergic neurotransmission, as already suggested in some reports ([16,21] and references therein). It is well known that the DA neurons in the striatum and limbic areas are under the control of GABAergic systems [3], and that the activity of the GABAergic neurons depends on dopaminergic activity, in such a way that an increase in the latter is associated with an increase in inhibitory GABAergic activity, at least in some brain areas [13]. Thus, the administration of amphetamine would increase GABAergic activity through a feedback mechanism, and that increase would be blocked by the administration of DALA, liberating the DA neurons from inhibitory GABAergic influences. On the other hand, when pimozide is injected together with DALA, this latter substance would be without effect, since the inhibition of DA receptors would by itself inhibit GABAergic activity. It has been shown, actually, that prolonged treatment with a neuroleptic causes an increase in GABA receptors in substantia nigra [12], supposedly due to a sustained reduction in the activity of GABAergic neurons. However, until further data have been obtained, the abovementioned arguments remain only plausible hypotheses.

It could be argued that DALA acts as a GABA receptor antagonist. This is unlikely since at least leucine-enkephalin is practically without activity as an inhibitor of GABA receptor binding, even at very high concentrations [14].

Finally, it could be maintained that DALA might be involved in the control of motility by its own, that is without interacting directly with dopamine or GABA. Our data don't support such a possibility, but it should not be completely discarded. However, it is more parsimonious to try to integrate the enkephalins with other transmitters known to participate in the control of locomotion.

To attribute an effect of a drug to an action on opiate receptors, it is usually required that this effect is inhibited by naloxone. The DALA + amphetamine induced stimulation of locomotion was only partially antagonized by naloxone. This may be due to the relatively low dose of naloxone used, and the short half-life of the drug *in vivo* (0.4 hr) [20]. Unfortunately, a higher dose of naloxone was not tried, because naloxone has effects on locomotion by itself. Nevertheless, it has been argued that even an antagonism by naloxone is not a sufficient proof of an action at opiate receptors [24]. We feel that the lack of affinity of enkephalins for other relevant receptors is more pertinent evidence as to the specificity of action.

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