Pharmacology Biochemistry & Behavior, Vol. 10, pp. 899–905 Printed in the U.S A

Effects of [Des-Tyr^1]-γ-Endorphin and α-Endorphin on Substantia Nigra Self-Stimulation

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(Received 3 February 1979)

DORSA, D. M., J. M. VAN REE AND D DE WIED. *Effects of [des-Tyr^1]-γ-endorphin and α-endorphin on substantia nigra self-stimulation.* PHARMAC. BIOCHEM. BEHAV. 10(6) 899–905, 1979 —The β-lipotropin fragments, [des-Tyr^1]-γ-endorphin (DTγE, β-LPH$_{62-77}$) and α-endorphin (β-LPH$_{61-76}$) affect self-stimulating behavior associated with electrical stimulation of neurons of the ventral tegmentum area of rats in an opposite way. Subcutaneous administration of DTγE (5 and 25 μg) attenuated and that of α-endorphin (5 and 25 μg) facilitated this behavior. Similar opposite effects were observed after subcutaneous treatment with respectively the neuroleptic haloperidol (5 μg) and the psychostimulant amphetamine (100 μg). By using a biphasic testparadigm of decreasing and subsequent increasing the stimulating current intensity it was noted that the neuropeptides predominantly exerted their effect on responding at current intensities in the neighbourhood of the threshold for eliciting the behavior, whereas the neuroleptic and psychostimulant drug appeared to affect responding at currents associated with maximal performance as well In contrast to haloperidol, the effectiveness of DTγE was of a long term nature, in that performance of the rat was still affected 24 hr after peptide treatment. The results support the hypothesis that DTγE in some aspects interacts with brain substrates in a way comparable to that of neuroleptics. The data further suggest that closely related fragments of β-lipotropin modulate on-going activity of in particular dopaminergic neuronal systems.

Intracranial self-stimulation Substantia nigra Dopamine Haloperidol Amphetamine
β-Endorphin fragments [Des-Tyr^1]-γ-endorphin α-Endorphin

A LARGE body of evidence has recently been accumulated which indicates that various peptide fragments of lipotropin (β-LPH) possess morphinomimetic properties (for references see [25]). These peptides have been shown to have numerous behavioral effects a number of which are not blocked by opiate receptor antagonists. Thus, the ability of β-endorphin (β-LPH$_{61-91}$), α-endorphin (β-LPH$_{61-76}$) and Met-enkephalin (β-LPH$_{61-65}$) to delay extinction of pole-jumping avoidance behavior is not prevented by naltrexone [8]. γ-Endorphin (β-LPH$_{61-77}$) was found to have an opposite effect to α-endorphin on extinction of pole-jumping avoidance behavior [9]. In fact, removal of the N-terminal tyrosine of γ-endorphin which eliminates its opiate-like activity yielded a peptide [des-Tyr^1]-γ-endorphin (DTγE, β-LPH$_{62-77}$), that appeared to be even more potent than γ-endorphin in facilitating the extinction of active avoidance behavior [9]. Further studies revealed that DTγE possesses a number of activities comparable to those of the neuroleptic drug haloperidol. The peptide however, appeared to be devoid of the typical locomotor and sedative effects of haloperidol [9].

Since the ability of neuroleptics to modify dopaminergic transmission is well known (for references see [33]), it was of interest to test the ability of DTγE to affect behaviors associated with activation of dopaminergic systems in the brain. For this reason, the present study deals with the action of DTγE on intracranial self-stimulation behavior (ICSS) associated with electrical stimulation of dopaminergic cell bodies of the ventral midbrain. In addition, the effects of this peptide were compared with those of α-endorphin, haloperidol and amphetamine.

METHOD

Animals

A total of 11 male Wistar rats (TNO, Zeist) weighing from 200–220 g at the time of implantation of electrodes were used. They were maintained on ad lib food and water and housed in separate cages throughout the experiment in a temperature controlled room on a 14 hour light-10 hour dark schedule. Five of the animals had been treated with peptides

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previously [10], but had been drug free for 2 weeks prior to testing in these experiments. Animals weighed during drug testing from 275–350 g.

Surgery

Each animal was implanted with a twisted bipolar stainless steel wire (150 μ) electrode in the area of the substantia nigra on the right side of the brain (Pellegrino and Cushman [16] coordinates A 2 2, L 2.0, D 3.5). The electrode was insulated except at a cross-section of the tip and fixed to the skull with anchor screws and dental cement. Surgery was performed using 0.1 ml Hypnorm® The animals were allowed to recover for one week following the operation before testing was initiated

Apparatus

The animals were trained and tested individually in an operant conditioning chamber (30×24×38 cm) with a Plexiglas front and back and a yellow light. Pressing one of two metal 5×2×1 cm levers positioned 6 cm above the grid floor delivered an 0.5 second biphasic square wave train of impulses through the electrode on a continuous reinforcement schedule. Each train consisted of impulses of a frequency 50 Hz, with a pulse width of 0.5 msec and a delay of 0.5 msec between the positive and negative pulses. The intensity of the stimulation was varied according to the experimental procedure and was repeatedly checked using an oscilloscope to measure the voltage drop across a 10 Kohm resistor. During the period of stimulation, the yellow light was turned off and pressing the lever did not produce an additional wave train.

General Test Procedure

The animals were shaped to self-stimulation in the experimental chamber. Training consisted of daily 10 minute sessions. Animals which failed to make more than 10 responses on the bar after 3 days training were considered non- or poor-self-stimulators and training of these animals was discontinued. The remaining animals were trained for 5 days at which time a maximal current intensity was established by increasing the stimulation intensity in steps of generally 80 μA to a level which elicited the maximal number of responses per 10 minute period. This current was used for daily training of the animals until stable response rates were obtained (variation less than $\pm$ 10% of the mean).

Animals were then subjected to a biphasic test paradigm. Current intensity was gradually decreased from the maximal (training) level to zero by steps of in general 80 μA (descending phase), and increased by the same steps to maximal current intensity again (ascending phase). The animals were exposed to each intensity for 4 minutes (sessions), but only the number of responses during the last 3 minutes of each session was recorded (responses per session). Based on the performance of the animals on 3 consecutive days, the current intensity at which more than 20 responses per session was achieved was established and a current of in general 40 μA below it was inserted into the paradigm. Bar pressing at the inserted current and the current 40 A below it was taken as the response rate at "around threshold" currents, i.e. that current at which the animal indicates by its behavior that it is able to distinguish that current from zero. Three trained impulses were given manually before the start of each session

Drug Testing

The animals were exposed in succession to maximum and "around threshold" current intensities (descending phase) and subsequent to "around threshold" and maximum current again (ascending phase of the biphasic test paradigm) for 4 consecutive days. Performance on the 4th day was taken as basal (Day 1) performance. On the next day (Day 2), the animals were treated with 0.5 ml of 0.9% NaCl (saline) solution administered subcutaneously 30 minutes prior to testing. The following day (Day 3) drug testing was performed by administering the test substance in 0.5 ml saline subcutaneously 30 minutes prior to testing. Performance of the animals on the following 2 days (Days 4 and 5) was also examined.

Five animals randomly selected were given 1, 5 and 25 μg doses of [des-Tyr^1]-γ-endorphin in that order. Animals were continuously trained in the biphasic test paradigm for at least 4 days between tests. The effects of α-endorphin (5 and 25 μg per rat), haloperidol (5 μg per rat), and amphetamine (100 μg per rat) were tested in 5 animals which were randomly selected. Each drug test was separated by at least 4 days training in the biphasic paradigm.

Data Analysis

Performance of the animals on Days 2, 3, 4 and 5 were expressed as a percent of baseline (Day 1) performance at each current intensity. Statistical comparisons of saline and drug effects were performed by Student's paired t-test.

Histology

Placement of the electrodes was examined histologically. Animals were anesthetized with pentobarbital, their chest cavities opened and saline followed by 10% Formalin was used to perfuse the brain via the left ventricle of the heart. The brains were then removed and placed in 4% Formalin. They were sectioned on a cryostat in 60 μ sections and stained with thionine blue and microscopically examined. The position of the electrodes was localized using the atlas of Pellegrino and Cushman [16].

Drugs and Peptides

All drugs and peptides were stored dry and diluted to appropriate concentrations immediately prior to use. [Des-Tyr^1]-γ-endorphin (H-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH. β-LPH$_{62-77}$) and α-endorphin (H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH: β-LPH$_{61-76}$) obtained from Organon International B.V., Oss, the Netherlands, were dissolved in saline. D-amphetamine (Dexamphetamini Sulfas, OPG, Utrecht, the Netherlands) was dissolved in saline and haloperidol (Janssen, Beerse, Belgium) in 0.1 ml 0.01 N tartaric acid and adjusted to a final volume of 0.5 ml with saline.

RESULTS

All animals which acquired self-stimulation behavior exhibited a characteristic motor side-effect in conjunction with each response. The animals showed contraversive body turning frequently accompanied by oscillations of the limbs on the left side of the body The severity of the motor effect was greatly reduced or disappeared entirely at low current intensities.

Figure 1 shows a typical response pattern obtained from

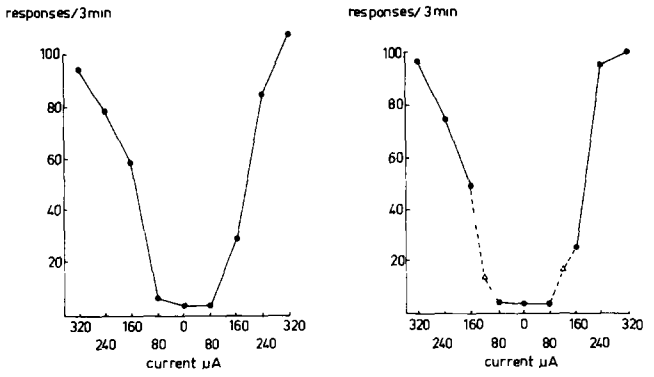

FIG. 1. Typical performance of an animal in the biphasic test paradigm. The animal was exposed for four minutes first to a current intensity which elicited a maximal response rate (320 μA). Current intensity was decreased by 80 μA steps to zero at 4 minute intervals, and was then increased to maximal again by the same increments. In the left panel, number of responses during the last three minutes of each session is plotted against current delivered. In the right panel is shown the animal's performance on the following day using the same test procedure except that a new "around threshold" current (120 μA) was inserted into the procedure (-----△-----).

an animal during the biphasic test paradigm. The number of responses per session was proportional to current intensity up to a maximum above which no further increase, or even a decrease in response rate was observed. The right panel in this figure shows performance of the same animal on the following day and its performance at the current intensity then inserted into the test paradigm. Current intensities which elicited maximal performance ranged from 50 to 400 μA. Maximal response rates on Day 1 were 26.8 ± 4.8 responses per min (mean ± SEM). Threshold currents were from 20 to 160 μA and responses on Day 1 were 6.6 ± 0.7 responses per min (mean ± SEM).

Figure 2 shows the effect of graded doses of DTγE on self-stimulation behavior as measured in the biphasic test paradigm. Subcutaneous administration of saline did not significantly modify the animals (N=5) performance at any current intensity tested. Administration of 1 μg DTγE also did not affect the behavior significantly as compared to saline treatment. DTγE, in a dose of 5 μg, however, significantly attenuated response rates at threshold current levels on the ascending phase of the test. There was also a small although significant ($p<0.05$) depression of responding at maximal performance levels during this phase. Twenty-five μg of DTγE had quite similar effects to 5 μg of the peptide. The performance of 3 of the 5 animals at threshold of the descending phase of the test was suppressed, but that of the other 2 animals was enhanced. Thus no significant effect was noted here. However, the performance of the animals in this portion of the test was significantly depressed when examined 24 hours after peptide administration. Likewise, threshold responding on the day of treatment and 24 hours later was significantly attenuated in the ascending phase. A small depression at maximal performance levels during the ascending phase was also noted on the day of treatment but not on the following day.

Figure 3 depicts the effects of α-endorphin on ICSS behavior. α-Endorphin in a dose of 5 μg significantly

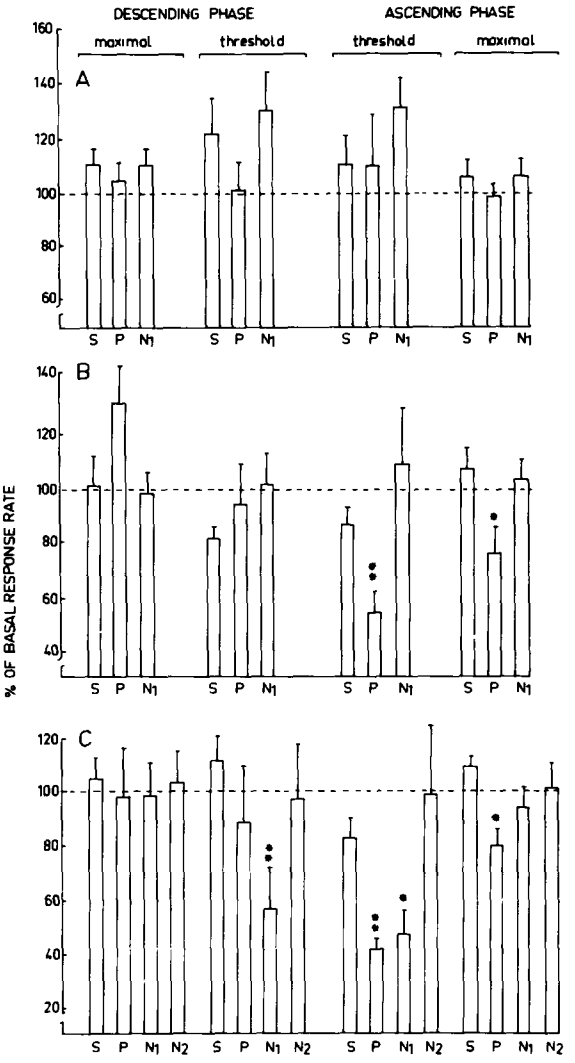

FIG. 2. Effect of [des-Tyr¹]-γ-endorphin (DTγE) on substantia nigra self-stimulating behavior using the biphasic test paradigm. Performance of the animals at maximal and threshold current intensities during the descending and ascending phase of the test paradigm are presented. All values are mean ± SEM of the response rate of animals (N=5) expressed as a percent of basal (Day 1) performance. The effects of subcutaneous treatment with 0 5 ml saline (S), 1 μg (part A), 5 μg (part B) or 25 μg (part C) DTγE in 0.5 ml saline (P), and no treatment on the 2 days following peptide administration (N_1 and N_2) are shown. Stars represent significant difference with respect to effects of saline treatment (*$p<0$ 05, **$p<0.01$)

enhanced responding at threshold current on the day of treatment. A tendency toward depression of responding ($p<0.1$ as compared to saline treatment) at threshold levels was observed on the day following treatment. The facilitation of ICSS after α-endorphin treatment was even more pronounced after administration of 25 μg of the neuropeptide. A tendency toward depression of the behavior ($p<0.1$ as compared to saline treatment) was noted on the day after treatment during the descending phase of the test paradigm. However, during the ascending phase an increased performance was observed, but this enhancement did not reach

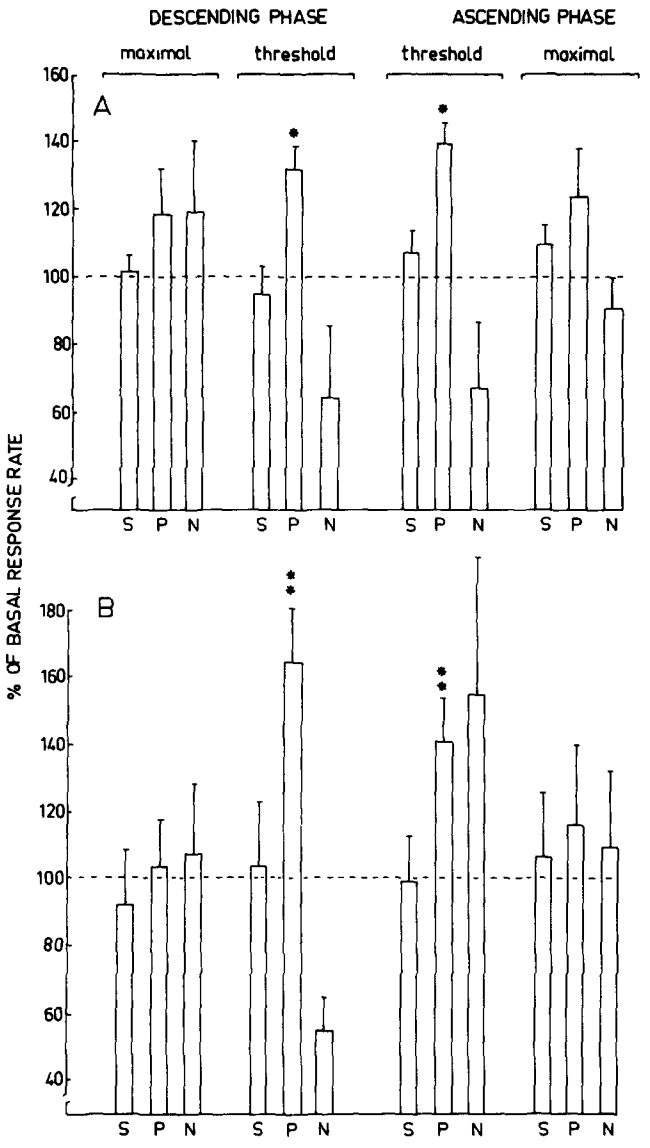

FIG. 3 Effect of α-endorphin on substantia nigra self-stimulating behavior using the biphasic test paradigm As in Fig 1, performance of animals (N=5) on the day given 0 5 ml saline (S), 5 μg (part A) or 25 μg (part B) α-endorphin in 0 5 ml saline (P) and no treatment (on the day after peptide testing, N) expressed as a percentage of basal performance (Day 1) are given (*$p<0$ 05, **$p<0$ 01 with respect to saline treatment)

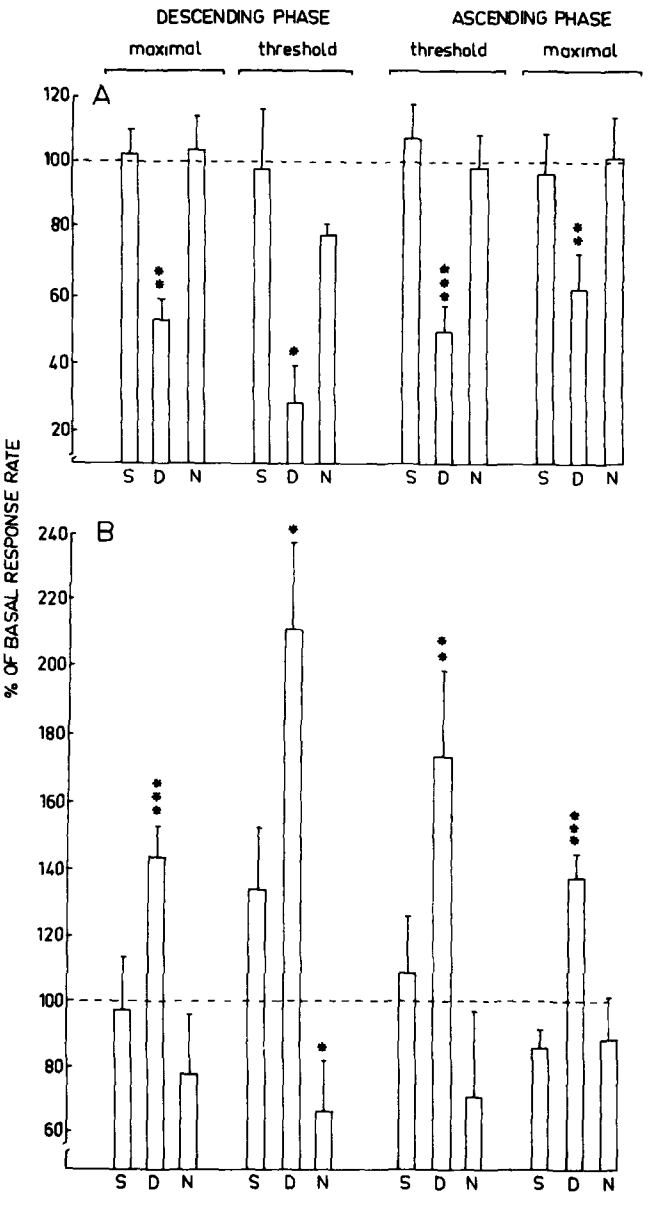

FIG. 4 Effect of haloperidol and amphetamine on substantia nigra self-stimulating behavior using the biphasic test paradigm. As in Fig 1, performance of animals (N=5) on the day given 0 5 ml placebo solution (S), 5 μg haloperidol dissolved in tartaric acid and saline (part A) or 100 μg d-amphetamine dissolved in saline (part B) and no treatment (on the day after peptide testing, N) expressed as a percent of basal performance (Day 1) are given (*$p<0.05$, **$p<0.02$, ***$p<0.01$ with respect to saline treatment)

statistical significance. Performance of the animals was not affected by α-endorphin at maximal current level on the day of treatment and the following day. Two days after peptide treatment the behavior of the animals at all current intensities was comparable to that observed after saline treatment.

Haloperidol in a dose of 5 μg (ca. 0.02 mg/kg) produced a significant decrease in response rates at both maximal and threshold current intensities (Fig. 4). This effect had disappeared 24 hours later. When animals were given 100 μg (ca. 0.3 mg/kg) d-amphetamine, enhancement of responding at all levels was noted. On the following day (Day 4 of testing) a reversal of effect occurred in that responding at threshold currents was depressed, but only significantly during the descending phase of the test. Performance of these animals on Day 5 was again comparable to that of the day of saline treatment

Histological examination of the brains of good and poor self-stimulators revealed that the animals which most easily acquired ICSS behavior from the ventral mesencephalon

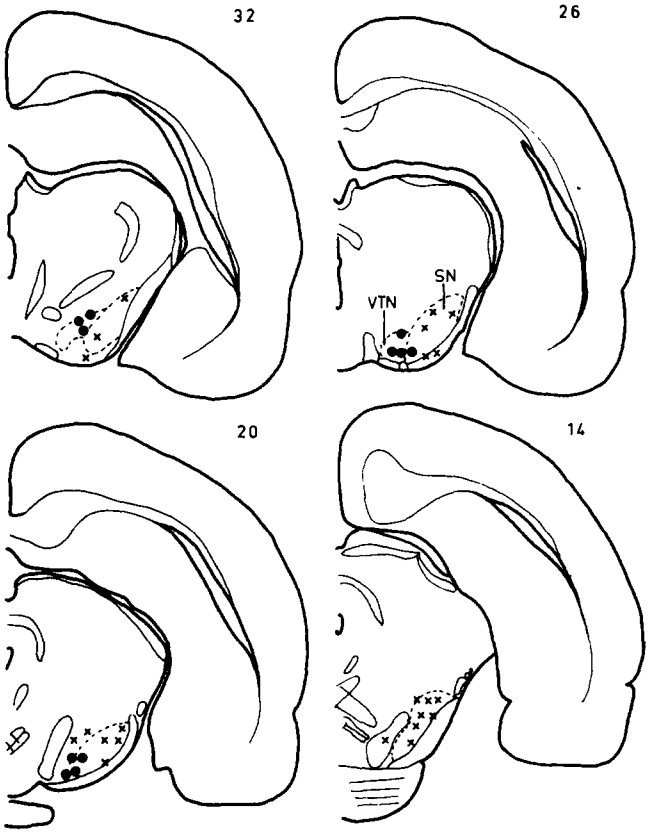

FIG. 5. Location of the tip of electrodes supporting (●) or not supporting (×) self-stimulation. For details, see Method. Sections correspond to the atlas of Pellegrino and Cushman [16]. SN=substantia nigra, VTN=ventral tegmentum nucleus.

have electrode placements which lie in the very medial portions of the substantia nigra and which may be in contact with cell bodies of the ventral tegmental area (Fig. 5). Animals with more lateral placements and those located more caudally in the nigral region were less likely to acquire the behavior.

DISCUSSION

The neurochemical and anatomical substrates of ICSS from the ventral mesencephalon have been a matter of some controversy over the last few years. In the present study, we find that animals will acquire self-stimulation behavior from electrodes implanted in the very medial portions of the substantia nigra and which impinges on the ventral tegmental area overlying the intrapeduncular nucleus. Crow [6] has reported similar findings in a more extensive study which mapped out effective sites in this region, and pointed out their proximity to dopaminergic cell bodies of the A10 cell group of Dahlström and Fuxe [7] and the pars compacta of the substantia nigra or A9 area [24]. The projections of these cells to innervate various regions of the forebrain are well known, but their importance in ICSS remains unclear. The ability of neuroleptic agents given peripherally [13,31] or directly into the striatum [4] to block ICSS has given rise to the notion of nigrostriatal involvement. However the depressant effect of such treatments on motor activity in general makes interpretation of such results difficult. Recently, Neill *et al.* [14] have presented evidence that mesolimbic projections from the medial nigra-ventral tegmentum to the ventral striatum and nucleus accumbens are critically involved in maintenance of ICSS from the lateral hypothalamus. A functional separation of this system from the traditionally thought of motor functions of the nigro-striatal pathway is suggested by these investigators. This is an attractive hypothesis, since dopamine has also been implicated as a mediator of lateral hypothalamic ICSS [31], but motor side effects similar to those seen from the nigral region are not observed with stimulation of this area. The body turning associated with nigral-ventral tegmental stimulation as observed in the present study has been described by others [1], and Szabo [23] found it to be an unavoidable contaminant to obtain ICSS behavior from this area.

Effects of various drugs on ICSS have been reported to be dependent upon the response rate at which they are tested [30]. The biphasic test paradigm used in this study allows two determinations of drug effects at maximal and low response rates within the same animal. In the present study, it was of particular interest to determine the effects of the tested neuropeptides at "around threshold" currents. It has been postulated that peptides act physiologically as neuromodulators in the brain [2]. If so, it might be expected that their effects would be more prominent at threshold current intensities which elicit submaximal response rates as has been reported to occur with ACTH like peptides [15].

Exogenously administered peptide fragments of the C-terminal portion of the β-lipotropin molecule modify intracranial self-stimulation (ICSS) behavior from electrodes implanted in the ventral mesencephalon. The depression of ICSS by [des-Tyr^1]-γ-endorphin (DTγE) administration is interesting in view of the similarity of this and other effects [9] of this peptide to those of neuroleptic agents such as haloperidol. Haloperidol has been reported to inhibit ICSS from the median forebrain bundle (MFB) [13,31], the locus coeruleus [21], the substantia nigra (SN) [4] and the nucleus accumbens [18]. The suppression due to DTγE, however, differed from that of haloperidol in its expression within the biphasic test paradigm used in the present study, as well as in duration of effect. The most striking effects of DTγE were observed when the rats were tested at around threshold currents, whereas haloperidol decreased the behavior also when maximal currents were delivered to the animals. Furthermore, the performance of the animals remained low when tested 24 hours after DTγE treatment while at that time animals treated with haloperidol showed responding comparable to saline treatment. Such effects would seem to suggest that DTγE is most probably not exerting its effects by simply blocking dopamine receptors critical for maintenance of ICSS as is suggested as the mode of action of haloperidol, since it is unlikely that a single peripheral injection of the peptide would allow receptor occupation to continue for more than 24 hours. In addition, the neuroleptic-like activities of DTγE are not related to an interaction with brain binding sites for neuroleptic agents since it possesses little ability to displace labeled neuroleptics from their brain binding sites as measured *in vitro* [26].

The facilitation of ICSS which occurred following α-endorphin administration was found to be similar but not identical to the effect of amphetamine. This psychostimulant drug has been reported to increase self-stimulation rates from electrodes implanted in the MFB, locus coeruleus and SN [5, 17, 18]. The amphetamine-induced increase of ICSS

behavior elicited from the SN is thought to result from activation of the dopaminergic systems arising from this nucleus through enhancement or release of functional dopamine [30,33]. However, it is unlikely that α-endorphin has a similar action in this respect, particularly in view of the differential effect of the peptide and of amphetamine on performance of the rats at maximal current intensity.

The mechanisms by which DTγE and α-endorphin exert their effects on ICSS behavior are not clear. Although α-endorphin has potentially opiate-like activity, this is not observed when it is administered peripherally in doses used in the present study ([11] and Van Ree, unpublished data). Thus, it is reasonable to assume that the effectiveness of α-endorphin on ICSS is not mediated by opiate receptors. α-Endorphin has been reported to affect avoidance behavior, which effect is also independent of brain opiate receptors [8]. Interestingly, DTγE has an effect on avoidance behavior opposite to that of α-endorphin [9]. Thus, the opposing effects of these two neuropeptides on ICSS bear a striking resemblance to their influence on avoidance behavior. Furthermore, it was found that haloperidol, like DTγE, facilitated extinction of active and attenuated retention of passive avoidance behavior, whereas both α-endorphin and amphetamine acted in an opposite way in both test paradigms [8, 9, 12]. Further experimentation is needed to elucidate the eventual relationship between the action of the peptides on ICSS and their influence on avoidance behavior.

Both DTγE and α-endorphin affect catecholamine (CA) activity in the brain. Versteeg *et al.* [29] have examined the influence of relatively low amounts of these peptides on NA and DA disappearance following α-methyl-para-tyrosine treatment in various microdissected brain regions. Only in a small number of brain areas catecholamine disappearance was affected. In general, CA disappearance was increased after DTγE treatment whereas α-endorphin decreased CA disappearance. Although these opposite effects may be related to the action of these peptides on ICSS behavior, neither DTγE nor α-endorphin affected CA disappearance in the area where the electrodes appeared to be located in the present study (ventral tegemental area-medial part of the substantia nigra). It has been argued before that the mesolimbic projections from the medial nigra-ventral tegmentum to the ventral striatum and nucleus accumbens may be critically involved in ICSS from these sites [6, 10, 14]. However, DTγE and α-endorphin did not affect DA nor NA disappearance in the nucleus accumbens. Although DA disappearance in the nucleus caudatus and globus pallidus was decreased after α-endorphin treatment, an opposite effect of DTγE was not observed. Thus, a clear relationship between the effectiveness of these peptides to modify SN self-stimulation and to affect CA disappearance can not be determined at present. It might be that the neuropeptides also interact with other neurotransmitter systems that influence dopaminergic activity. For example, evidence has been presented that enkephalinergic systems are involved in ICSS [3], particularly in that elicited from the SN [22] and for a transsynaptic regulation of DA by enkephalin containing neurons [19,20].

In the present study, the effects of the neuropeptides, although similar with respect to the direction of effect, were quite different from that of the reference drugs. In general, the neuropeptides modified ICSS behavior only when the animals were tested at around threshold current intensity, while the drugs were also active at maximal current. It has been argued that neuropeptides act physiologically as neuromodulators in the brain [2]. Such a modulating role by which these entities promote subtle alterations in on-going activity of various neural systems, might explain that the effectiveness of the tested peptides on ICSS are more prominent at threshold current intensities which elicit submaximal response rates. The interaction between neuropeptides and ICSS may be rather complex, since it has been shown that neuropeptides related to ACTH [15], vasopressin and oxytocin [10] also interfere with ICSS behavior. Moreover, closely related peptides, i.e., DTγE and α-endorphin which have 15 of their 16 amino acids in common, affect ICSS behavior in an opposite way, as was already noted before with respect to the action of these neuropeptides on avoidance behavior [9]. However, the similarity of effect of DTγE and haloperidol as described in this study further substantiates the hypothesis that DTγE has potential neuroleptic-like or antipsychotic activity [9, 27, 28].

ACKNOWLEDGEMENT

This research was supported by a grant of the Stichting Farmacologisch Studiefonds, Utrecht, The Netherlands

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