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EFFECTS OF (Des-Tyr^1)-γ-ENDORPHIN AND α-ENDORPHIN AS COMPARED TO HALOPERIDOL AND AMPHETAMINE ON NUCLEUS ACCUMBENS SELF-STIMULATION

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Summary—The β-endorphin fragment (Des-Tyr^1)-γ-endorphin (DTγE, β-LPH 62–77) attenuated self-stimulation behaviour associated with electrical stimulation of the nucleus accumbens area of rats. This effect was observed after subcutaneous (s.c.) injection of 2.5 and 25 μg of the neuropeptide and appeared to be dose-dependent. It was present only at current intensities in the neighbourhood of the threshold for eliciting the behaviour. Subcutaneous administration of haloperidol (5 μg) exerted a similar effect, but in addition affected the rate of responding at currents associated with maximal performance. Amphtamine (100 μg, s.c.) enhanced responding at both low and high current intensities. α-Endorphin (β-LPH 61–76; 2.5 and 25 μg s.c.) did not influence nucleus accumbens self-stimulation behaviour.

It is postulated that DTγE affects this behaviour by interfering with mesostriatal dopaminergic neuronal systems projecting to the nucleus accumbens, possibly via an action on synaptic membranes.

In a number of behavioral testparadigms it was observed that the non-opiate-like fragment of β-endorphin, Des-Tyrosine^1-γ-endorphin (β-LPH 62–77, DTγE), possesses a number of activities comparable to those of the neuroleptic drug haloperidol (De Wied, Kovács, Bohus, Van Ree and Greven, 1978). Moreover, α-endorphin (β-LPH 61–76) has behavioural activities which in some aspect resemble those of the psychostimulant drug amphetamine (Van Ree, Bohus and De Wied, 1980). Detailed analysis revealed that the peptides do not mimic all effects of the drugs, in particular, those on gross behavior (De Wied *et al.*, 1978, Van Ree *et al.*, 1980, Weinberger, Arnsten and Segal, 1979). In addition, DTγE does not displace [^{3}H]-haloperidol, [^{3}H]-spiperone or [^{3}H]-apomorphine from their stereospecific binding sites in brain membrane preparations, as found with haloperidol (Van Ree, Witter and Leysen, 1978, Van Ree *et al.*, 1980; Pedigo, Ling, Reisine and Yamamura, 1979).

These findings have led to the postulate that DTγE may be beneficial in psychotic patients, but with a profile more specific than that of currently used neuroleptic drugs (De Wied *et al.*, 1978; De Wied, 1978). Indeed, the first clinical studies on schizophrenic patients showed an antipsychotic effect of DTγE treatment (Verhoeven, Van Praag, Van Ree and De Wied, 1979).

The behavioural effects of haloperidol and other neuroleptics are assumed to be produced through dopaminergic systems by blocking dopaminergic receptors (Andén, Butcher, Corrodi, Fuxe and Ungerstedt, 1970; Wauquier, 1976). Amphetamine is known to release monoamines, including dopamine, from presynaptic storage sites and this action may be responsible for the behavioural effects of this drug (Von Voightlander and Moore, 1973; Wise, 1978). This hypothesis stimulated research on the interaction of DTγE and α-endorphin with behaviours associated with activation of dopaminergic systems in the brain in order to investigate whether these peptides have a similar mode of action to haloperidol and amphetamine on these behaviours. The first studies in this respect deal with electrical self-stimulation behaviour of the ventral tegmental substantia nigra area which, among others, contains the dopaminergic cell bodies of the mesostriatum dopaminergic pathway, projecting to the nucleus accumbens (Dorsa, Van Ree and De Wied, 1979; Lindvall and Björkland, 1978).

It was found that this behaviour was attenuated by DTγE and haloperidol and facilitated by α-endorphin and amphetamine. In the present study the effects of these peptides and drugs have been examined on intracranial self-stimulation behaviour associated with electrical stimulation of the nucleus accumbens area, which contains dopaminergic terminals of the mesostriatum dopaminergic pathway originating from the ventral tegmental area (Lindvall and Björkland, 1978).

Key words: electrical self-stimulation, nucleus accumbens, (Des-Tyr^1)-γ-endorphin, α-endorphin, amphetamine, haloperidol, β-endorphin fragments.

METHODS

Animals

A total of 26 male Wistar rats (TNO, Zeist) weighing from 190 to 210 g at the time of implantation were

used. They were kept under standard conditions (room temperature 22 ± 1°C, light on from 5 a.m. till 7 p.m.) and housed in separate cages with *ad libitum* access to food and water. At the time of testing the animals weighed from 275 to 350 g.

Surgery

Each animal was implanted with a twisted bipolar stainless-steel wire (150 μ) electrode in the nucleus accumbens area on the right side of the brain (coordinates according to Pelligrino and Cushman, 1967: A: 9.0, L: 0.5, D: 1.6). Surgery was performed using 0.1 ml Hypnorm®. The electrode was insulated except at a cross-section of the tip and fixed to the skull with anchor screws and dental cement. The animals were allowed to recover for at least one week following the operation before testing was initiated.

Apparatus

The animals were trained and tested individualy in an operant conditioning chamber as described in detail previously (Dorsa *et al.*, 1979). Briefly, pressing one of the two levers was followed by an 0.5 sec biphasic square wave train of impulses through the electrode on a continuous reinforcement schedule. Each train consisted of impulses of a frequency 70 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.

General test procedure

The animals were shaped and trained to self-stimulation during 10 min sessions daily. Animals which made more than 10 responses in the box after 3 days training were considered good stimulators. The other rats were regarded as non- or poor self-stimulators and training of these animals was discontinued. The good stimulators were subsequently trained for one week. During this period the current which elicited a maximal number of responses per session, was established. This current was used for further training until the animals showed stable response rates (variation less than 10% of the mean). The animals were then subjected to a biphasic test paradigm as described previously in detail (Dorsa *et al.*, 1979). Briefly, current intensity was decreased from the maximal (training) level to zero by steps of (depending on the maximal current) 80 or 40 μA (descending phase) and increased by the same steps to maximal current again (ascending phase). The animals were tested on each intensity for 4 min (session). The responses during the last 3 min of each session were recorded (responses per session). The current intensity at which more than 20 responses per session were performed during 3 consecutive days was established and a current of 40 or 20 μA (depending on the sensitivity of the animal) below it was inserted into the paradigm. The performance at the inserted current and the current 40 or 20 μA below it was taken as the response at threshold currents. Three trained impulses were given manually before the start of each session.

Drug testing

The rats were tested in succession on maximum and "threshold" current intensities (descending phase) and subsequently on "threshold" and maximum current (ascending phase of the biphasic testparadigm). After training to stable response rates in this paradigm, the animals were tested weekly for 5 consecutive days. On the second day the animals were injected subcutaneously with 0.5 ml of 0.9% NaCl (saline) solution 30 min prior to testing. The following day (day 3) drug or peptide was administered subcutaneously 30 min prior to testing. No treatment was given on the first, fourth and fifth day of testing. Five animals, randomly selected, were injected with 5 μg haloperidol, 100 μg amphetamine, 2.5 and 25 μg (Des-Tyr^1)-γ-endorphin and 2.5 and 25 μg α-endorphin.

Data analysis

Performance of the animals on days 3, 4 and 5 were expressed as a percent of that on day 2 at each current intensity. Statistical analysis was performed using Student's paired *t*-tests.

Histology

After completion of the experiments, the placements of the electrodes were examined histologically as described elsewhere (Dorsa *et al.*, 1979). The position of the electrodes were localized using the atlas of Pellegrino and Cushman (1967).

Drugs and peptides

Haloperidol (Janssen, Beerse, Belgium) was dissolved in 0.1 ml 0.01 N tartaric acid and adjusted to a final volume of 0.5 ml with saline and D-amphetamine (Dexamphetamine Sulfas, OPG, Utrecht, The Netherlands) was dissolved in saline. (Des-Tyr^1)-γ-endorphin (β-LPH 62-77, DTγE) and α-endorphin (β-LPH 61-76), obtained from Organon International BV, Oss, The Netherlands, were dissolved in saline. Drugs and peptides were stored dry and their solutions were prepared immediately prior to use.

RESULTS

Nine out of 26 animals appeared to be good self-stimulators. The rate of self-stimulation changed as a function of current intensity: decreasing the current led to a lower rate and increasing the current enhanced response rate. Maximal performance was obtained with current intensities ranging from 60 to 400 μA. On day 1 maximal response rates were 16.4 ± 1.7 responses/min (mean ± SEM). Threshold currents were from 20 to 200 μA and responses on day 1 were 8.1 ± 1.0 responses/min (mean ± SEM). Saline treatment did not significantly modify the performance of the animals at any current intensity tested.

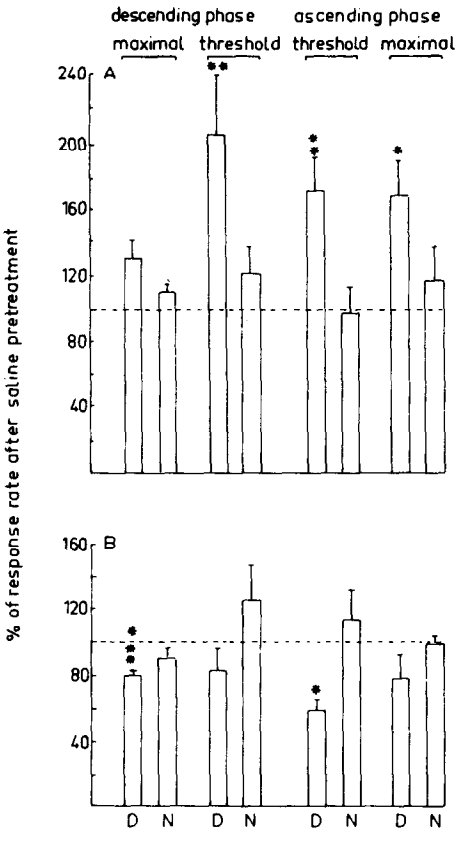

Fig. 1. Effects of 100 μg amphetamine (A) and 5 μg haloperidol (B) administered subcutaneously on nucleus accumbens self-stimulation behaviour using the biphasic test paradigm. Performance of the animals at maximal and threshold current intensities during the descending and ascending phase of the test paradigm are presented. All values are mean ±SEM of the response rate of animals ($n = 5$) expressed as a percent of performance after saline pretreatment on the day before drug (D) testing. The performance of the animals on the day (N) after that of drug treatment is also shown. (*$P < 0.05$, **$P < 0.02$, ***$P < 0.01$, with respect to saline treatment.)

The effects of amphetamine and haloperidol are shown in Figure 1. Amphetamine enhanced response rates at both maximal and threshold current intensities. On the day following treatment, the responding of the animals was not different from that on day 2. Haloperidol produced a significant decrease in response rate at maximal as well as threshold current intensities. Performance of the animals on the day following haloperidol treatment was comparable to that of the day of saline treatment.

Figure 2 depicts the effect of DTγE on self-stimulation behaviour. This peptide attenuated the response rate of the animals when they were tested at threshold current intensities during the ascending phase of the biphasic test paradigm. This effect appeared to be dependent on the dose of the peptide. On the day following treatment the performance of the animals was not different from that after saline treatment on day 2. In contrast to DTγE, neither 2.5 nor 25 μg of α-endorphin affected self-stimulation behaviour (Fig. 3).

Histological examination of the location of electrodes of good and poor self-stimulators revealed that animals which easily acquired self-stimulation behaviour have electrode placements in or close to the nucleus accumbens (Fig. 4).

DISCUSSION

Evidence for the role of dopaminergic systems in brain stimulation reward has been presented during recent years. Dopamine antagonists have been reported to inhibit self-stimulation from the medial forebrain bundle (Lippa, Antelman, Fisher and Canfield, 1973; Wauquier and Niemegeers, 1972), the locus coeruleus (Sandberg and Segal, 1978), the substantia nigra (Broekkamp and Van Rossum, 1975) and the nucleus accumbens (Philips, Brooke and Fibiger, 1975). However, until recently it has not been clearly demonstrated whether these drugs affect behaviour by interfering with reward mechanisms or with motor performance in general (Fibiger, 1978;

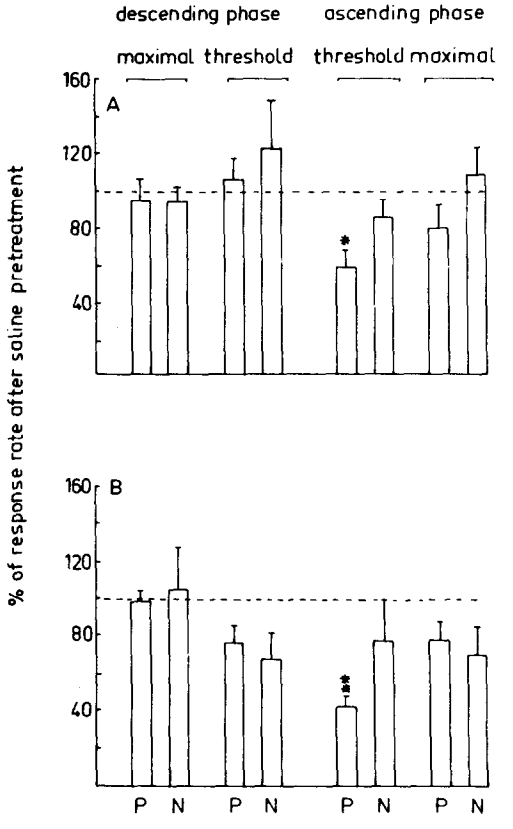

Fig. 2. Effects of 2.5 μg (A) and 25 μg (B) of (Des-Tyr[1])-γ-endorphin (DTγE) administered subcutaneously on nucleus accumbens self-stimulation behaviour using the biphasic test paradigm. For details see legend to Figure 1. (P = peptide; N = day after peptide treatment, *$P < 0.05$, **$P < 0.01$ with respect to saline treatment on the day before peptide treatment.)

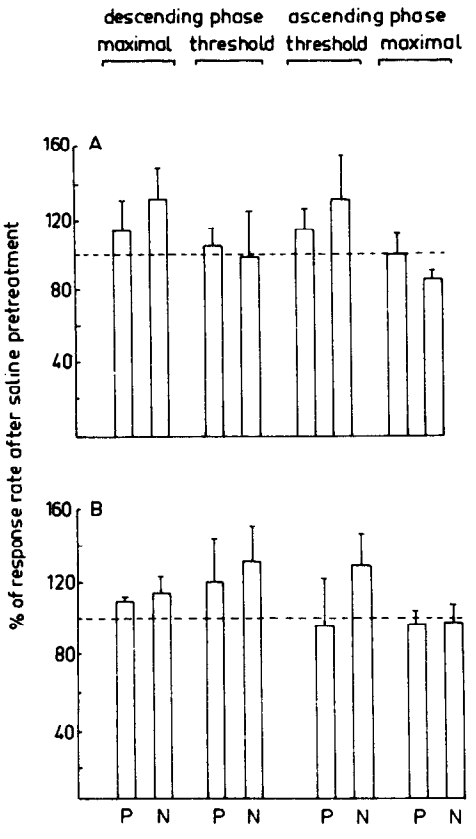

Fig. 3. Effect of 2.5 μg (A) and 25 μg (B) of α-endorphin administered subcutaneously on nucleus accumbens self-stimulation behaviour using the biphasic test paradigm. For details see legend to Figure 1 (P = peptide; N = day after peptide treatment.)

Wise, 1978). Mogenson, Takigawa, Robertson and Wu (1979) showed that injection of the dopamine antagonist, spiroperidol, into the nucleus accumbens reduced self-stimulation of the ipsilateral ventral tegmental area but did not influence self-stimulation of the contralateral ventral tegmental area. Similar effects were observed with nucleus accumbens self-stimulation. In addition, it was found that spiroperidol, injected into the frontal cortex, did not affect self-stimulation of the ventral tegmental area. These observations provide evidence that dopaminergic neurons of the mesostriatal dopaminergic pathways, projecting to the nucleus accumbens, are involved in self-stimulation of the ventral tegmental area and the nucleus accumbens. On the other hand, injection of the dopamine antagonist haloperidol into the caudatus-putamen, attenuated self-stimulation of the midbrain tegmentum when this drug was applied ipsilateral or contralateral of the stimulated site (Broekkamp and Van Rossum, 1975). Thus, the effects on the dopaminergic neurones projecting to the nucleus caudatus-putamen may be more related to motor performance than to reward mechanisms.

Previous studies have shown that subcutaneous administration of the β-endorphin fragment (Des-Tyr^1)-γ-endorphin (DTγE, β-LPH 62–77) or haloperidol attenuated, and that of α-endorphin (β-LPH 61–76) or amphetamine increased, self-stimulation of the ventral tegmental area (Dorsa *et al.*, 1979). Although the neuropeptides acted in a similar way to the drugs with respect to the direction of the effect, the behaviour was modified, particularly when the rats were tested at around threshold current intensity, while the drugs were also active at maximal current. The present data show that subcutaneous administration of haloperidol decreased, and that of amphetamine enhanced, the rate of self-stimulation of the nucleus accumbens of both maximal and threshold current intensities. The effects were similar to those found with self-stimulation of the ventral tegmental area, although haloperidol was somewhat less effective. This may be evidence that other systems than dopaminergic neuronal systems may be concerned in nucleus accumbens self-stimulation, when compared with that

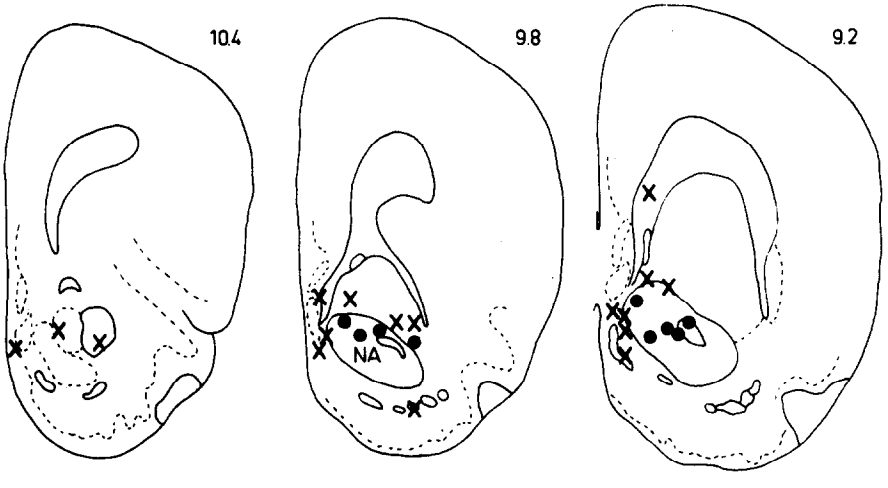

Fig. 4. Location of the tip of electrodes supporting (●) or not supporting (×) self-stimulation. Sections correspond to the atlas of Pellegrino and Cushman (1967). NA = nucleus accumbens.

of the ventral tegmental area. Self-stimulation of the nucleus accumbens was attenuated by DTγE in a way similar to that observed following stimulation of the ventral tegmental area, in that responding at threshold currents during the ascending phase of the biphasic test paradigm was affected. As in the case of haloperidol, the potency of DTγE in affecting nucleus accumbens self-stimulation was somewhat less when compared to that of the ventral tegmental area. These data may suggest that DTγE influences nucleus accumbens self-stimulation by blocking dopaminergic activity in this region. This is supported by the finding that DTγE implanted into the nucleus accumbens mimics the action of ergometrine, an antagonist of dopaminergic receptors in that region (Cools, 1977). This antagonist blocks the excessive grooming response following intraventricular administration of $ACTH_{1-24}$ (Bohus and Gispen, 1978). That DTγE may act on postsynaptic elements of dopaminergic neurones is supported by data showing that this neuropeptide, like haloperidol, counter-acts amphetamine-induced attenuation of the facilitated grasping response of rats with lesions in the parafascicular area of the thalamus (Van Ree *et al.*, 1980). However, the mode of action of DTγE might be quite different from that of haloperidol. In contrast to haloperidol, DTγE possesses little or no ability to displace labelled neuroleptics from their stereospecific binding sites located in the nucleus accumbens area as measured *in vitro* (Van Ree *et al.*, 1978; Pedigo *et al.*, 1979). Thus, the exact nature of the presumed interference of DTγE with postsynaptic membranes of dopaminergic neurones is as yet unknown.

A quite different picture emerges with respect to α-endorphin. This neuropeptide enhanced the rate of self-stimulation of the ventral tegmental area, when the animals were tested at threshold currents. However, α-endorphin did not affect the nucleus accumbens self-stimulation. Since amphetamine increased self-stimulation elicited from both areas, it seems likely that the site of action of α-endorphin differs from that of amphetamine. Thus, α-endorphin may exert its effect on electrical self-stimulation of the ventral tegmental area by an action on dopaminergic cell bodies in this region or by changing the activity of these neurons via a transsynaptic mechanism.

In conclusion, the present data provide evidence that the fragments of β-endorphin, DTγE and α-endorphin affect the activity of the mesolimbic dopaminergic projection to the nucleus accumbens. Since these peptides are present in rat brain and pituitary (Verhoef, Loeber, Burbach, Witter and De Wied, unpublished), in human cerebrospinal fluid (Loeber, Verhoef, Burbach and Van Ree, 1979) and guinea-pig ileum (Opmeer, Loeber and Van Ree, unpublished) and that either DTγE or α-endorphin may be selectively formed from β-endorphin by enzyme systems in brain synaptosomal preparations (Burbach, Loeber, Verhoef, De Kloet, 1980), it might be postulated that these neuropeptides play a physiological role in reinforcing mechanisms mediated by mesostriatal dopaminergic systems projecting to the nucleus accumbens. Moreover, the presumed neuroleptic and antipsychotic effects of DTγE may be related to an action of this neuropeptide or a metabolite on pre- or postsynaptic membranes of dopaminergic neurones in the nucleus accumbens area, which may be involved in the beneficial effects of neuroleptics in psychotic patients (Crow, Johnstone, Longden and Owen, 1978).

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