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The hypomotility elicited by small doses of apomorphine seems exclusively mediated by dopaminergic systems in the nucleus accumbens

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The reduction of motor activity elicited in rats by a subcutaneous injection of a small dose of apomorphine was reversed by pretreatment of the nucleus accumbens with haloperidol (10 pg), sulpiride (10 pg) or desenkephalin-γ-endorphin (DEγE) (100 pg or 10 ng). These doses of the compounds did not change motor activity in placebo-treated rats. Pretreatment of the nucleus caudatus with the same neuroleptics or DEγE did not diminish the effect of subcutaneously administered low doses of apomorphine. A small dose of apomorphine decreased motor activity when it was injected directly into the nucleus accumbens. This effect was dose dependently antagonized by subcutaneous pretreatment with DEγE. It is suggested that the hypoactivity elicited by small doses of apomorphine is exclusively mediated by dopaminergic systems in the nucleus accumbens.

Apomorphine; Locomotor activity; Nucleus accumbens; Nucleus caudatus; Desenkephalin-γ-endorphin; Neuroleptics

1. Introduction

Apomorphine and other dopamine (DA) receptor agonists have a biphasic effect on various behaviours in rodents; low doses produce decreased motor activity and high doses produce hyperactivity and stereotypy (DiChiara et al., 1976; Strömbom, 1976). These opposing effects have been explained by the postulate that DA agonists act at different DA receptors to induce these effects i.e. at presynaptically located DA autoreceptors and postsynaptic DA receptors respectively (Strömbom, 1976; Skirboll et al., 1979; Costall et al., 1981). These behavioural effects of apomorphine occur not only after subcutaneous (s.c.) treatment but also after local injection into the nucleus accumbens area of the brain. Thus, injections of 1 or 10 ng apomorphine into the nucleus accumbens decreased motor activity (Van Ree and Wolterink, 1981), while a dose of 10 μg increased motor activity (Van Ree et al., 1983). The hypoactivity induced by s.c. treatment with small doses of apomorphine can be antagonized by s.c. administration of the classic neuroleptic haloperidol, the atypical neuroleptic sulpiride or the neurolepticum-like γ-type endorphins i.e. des-Tyr^1-γ-endorphin (DTγE, β-endorphin (βE)-(2-17)) and desenkephalin-γ-endorphin (DEγE, βE-(6-17)) (Van Ree et al., 1982b; Serra et al., 1983; Ståhle and Ungerstedt, 1986). The same antagonism has been reported to follow local injections of apomorphine and these neuroleptic drugs and neuroleptic-like peptides into the nucleus accumbens (Van Ree et al., 1982a). These data suggest that the dopaminergic systems mediating the hypoactivity of s.c. injected apomorphine could be localized in the nucleus accumbens.

This hypothesis was tested in the present ex-

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periments dealing with s.c. and intracerebral administration of substances in the same animal. The data show that the hypomotility elicited by small doses of apomorphine seems exclusively mediated by dopaminergic systems in the nucleus accumbens.

2. Materials and methods

2.1. Animals and surgical procedure

Male Wistar rats of an inbred strain (TNO, Zeist, The Netherlands), weighing 130-150 g at the time of operation, were used. They were kept under standard conditions (room temperature 22 $\pm$ 1°C, light on from 5:00 a.m. till 7:00 p.m.), were housed in groups of 5 and received food and water ad libitum. The rats were anaesthetized with Hypnorm® (1 ml/kg body weight) and were secured in a stereotaxic instrument. Stainless steel cannulas (0.6 mm outer diameter, 0.3 mm inner diameter) were implanted on each side of the brain and were aimed at the nucleus accumbens or nucleus caudatus respectively (Van Ree and Wolterink, 1981; Van Ree et al., 1983). The coordinates for implantation into the nucleus accumbens area were 2.6 mm anterior to the bregma, 2.7 mm lateral to the midline, 6.1 mm below the dura, according to Pellegrino and Cushman (1967) except that the level of the upper incisor bar was at the level of the interaural line and the cannulas were inserted at an angle of 12°. The coordinates for implantation into the nucleus caudatus area were 0.3 mm anterior to the bregma, 3.8 mm lateral to the midline and 5.2 mm below the dura and the cannulas were inserted at an angle of 10°. The rats were allowed to recover from the operation for at least 6 days. The sites of injection were evaluated histologically as described before (Van Ree and Wolterink, 1981). Data from rats with cannulas outside the designated area were discarded from the analyses.

2.2. Behavioural procedure

Experiments were carried out between 8:00 a.m. and 5:00 p.m. in a sound-attenuated room. Two injections were given, spaced by a time interval of 40 min; a neuroleptic drug, DEγE or placebo was injected first (pretreatment) followed by an injection with apomorphine or placebo (treatment). The rats were injected bilaterally with 1 μl in a Hamilton syringe and a needle (0.25 mm outer diameter) inserted into the guide cannula. The rats were injected s.c. in the neck. The rats were placed in a rectangular perspex observation cage (bottom 20 $\times$ 6.5 cm, height 32 cm) 5 min after the last injection and locomotor activity was measured for 3 min by counting the number of crossings over the midline of the floor. The rats were tested again at 20 min after the last injection in a circular perspex test cage (diameter 19.5 cm, height 28.5 cm), the bottom of which was divided into 4 equal sections. Motor activity in this small open field was measured for 3 min by counting the number of sections explored at least with the forelegs. In the experiment in which DEγE was given s.c., the animals were tested once in the small open field 20 min after the last injection.

2.3. Drugs and peptides

Apomorphine (apomorphine HCl), haloperidol (Haldol®) and sulpiride (Dogmatil®) were obtained from O.P.G. Utrecht, Hypnorm® (10 mg/ml fluanisone, 0.2 mg/ml fentanyl base) from Duphar B.V., Amsterdam, The Netherlands. Desenkephalin-γ-endorphin (DEγE, βE-(6-17)) was kindly donated by Organon International B.V., Oss, The Netherlands. Apomorphine and DEγE were dissolved in saline immediately prior to use. The volumes used were 0.3 ml or 1 μl for s.c. or intracerebral injection respectively. Placebo treated animals received a similar volume of saline (0.9% NaCl). Haldol® and Dogmatil® were diluted with saline to the appropriate concentration.

2.4. Data analysis and statistics

Groups means and S.E. were calculated. The data were first analysed using a one-way analysis of variance (ANOVA) then with the Newman-Keuls procedure when the outcome revealed a statistically significant effect ($P < 0.05$).

3. Results

3.1. Injection sites

The sites of injection aimed at the nucleus accumbens appeared to be bilateral and in the middle and anterior part of the nucleus accumbens. In the nucleus caudatus the sites were found in the middle part (fig. 1).

3.2. Systemically injected apomorphine and pretreatment in the nucleus accumbens

Two experiments were performed. The first experiment concerned the effect of pretreatment of the nucleus accumbens with 10 ng DEγE on the behavioural effects elicited by a s.c. injection of a low dose of apomorphine (62.5 μg/kg) (fig. 2). Apomorphine significantly decreased motor activity in both test cages while DEγE completely antagonized this effect of apomorphine (ANOVA: $F(3,25) = 4.4$ ($P < 0.05$) and $F(3,25) = 12.9$ ($P < 0.001$) with respect to the rectangular test box and the small open field respectively). DEγE did not change motor activity in placebo-treated rats. In the second experiment the effect of pretreatment with 100 pg DEγE or 10 pg haloperidol or sulpiride

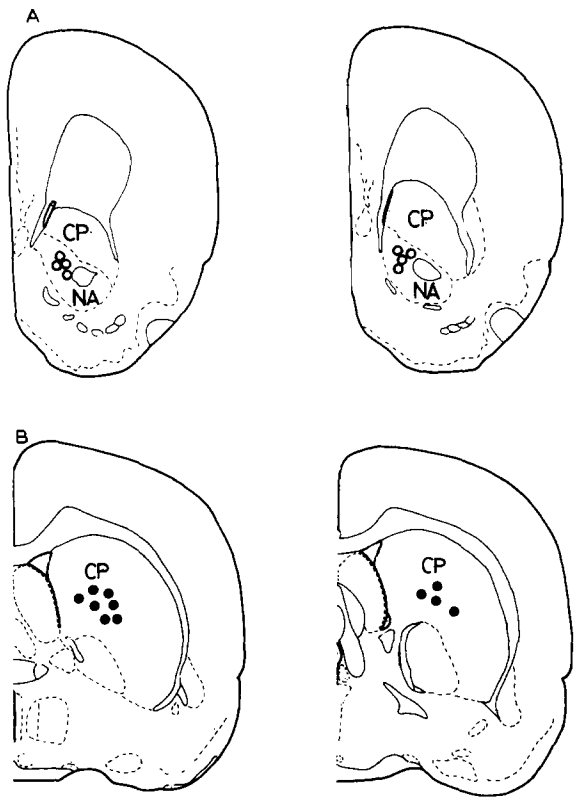


Fig. 1. Position of the tip of the cannulas in the nucleus accumbens (○) and nucleus caudatus (●) as revealed by histological evaluation. Ten representative tips are indicated for each injection area in drawings taken from Pellegrino and Cushman (1967); NA = nucleus accumbens, CP = nucleus caudatus.

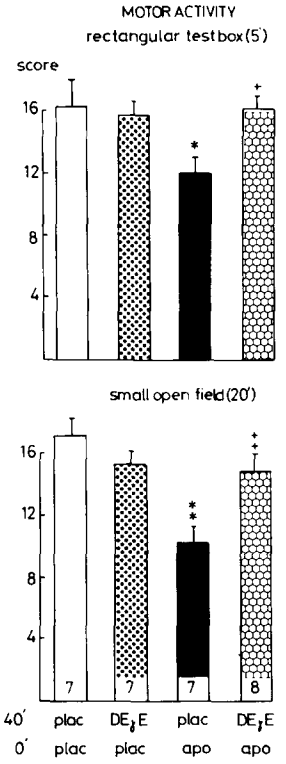
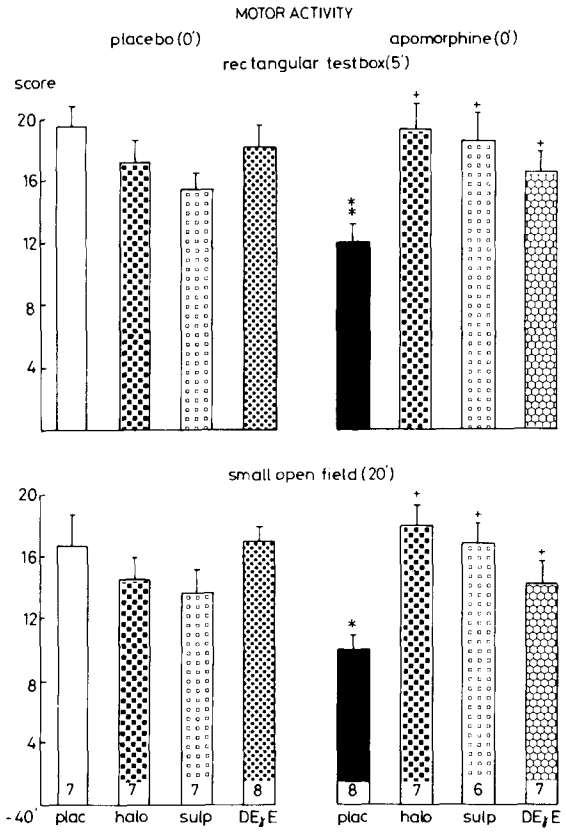


Fig. 2. The influence of pretreatment of the nucleus accumbens with DEγE on the apomorphine-induced hypoactivity when apomorphine was given s.c. Motor activity was measured for 3 min at 5 min (rectangular test box) and 20 min (small open field) after injection with placebo (saline) or apomorphine (62.5 μg/kg). Animals were pretreated in the nucleus accumbens with placebo (1 μl saline) or DEγE (10 ng) 40 min before the s.c. injection of placebo or apomorphine. The mean score for motor activity per treatment group is presented. Vertical bars indicate S.E.M. The number of animals per group is given in the columns. Different from placebo, placebo-treated rats (* $P < 0.05$, ** $P < 0.001$). Different from placebo, apomorphine-treated rats ($^{+}$ $P < 0.05$, $^{++}$ $P < 0.005$).

was tested on the behavioural effects induced by a s.c. injection of apomorphine (fig. 3). Pretreatment with DEγE, haloperidol or sulpiride of the nucleus accumbens 40 min prior to the s.c. apomorphine injection completely prevented the apomorphine-induced decrease of motor activity in both test cages (ANOVA: $F(3,24) = 5.3$ ($P < 0.01$) and $F(3,24) = 10.3$ ($P < 0.001$) with respect to the rectangular test box and small open field respectively). The neuroleptics and DEγE did not affect motor activity in placebo-treated rats (ANOVA: $F(3,25) = 1.9$ and $F(3,25) = 1.4$ with respect to the rectangular test box and small open field respectively). Sulpiride caused a slight, though not significant reduction of motor activity.



Fgi. 3. The influence of pretreatment of the nucleus accumbens with DEγE and neuroleptics on the apomorphine-induced hypoactivity when apomorphine was given s.c. Motor activity was measured for 3 min at 5 min (rectangular test box) and 20 min (small open field) after injection with placebo (saline) or apomorphine (62.5 μg/kg). Animals were pretreated in the nucleus accumbens with placebo (plac) (1 μl saline) or with haloperidol (halo, 10 pg), sulpiride (sulp, 10 pg) or DEγE (100 pg) 40 min before the s.c. injection of placebo or apomorphine. The mean score for motor activity per treatment group is presented. Vertical bars indicate S.E.M. The number of animals per group is shown in the columns. Different from placebo, placebo-treated rats (* $P < 0.005$, ** $P < 0.001$). Different from placebo, apomorphine-treated rats ($^{+}$ $P < 0.05$).

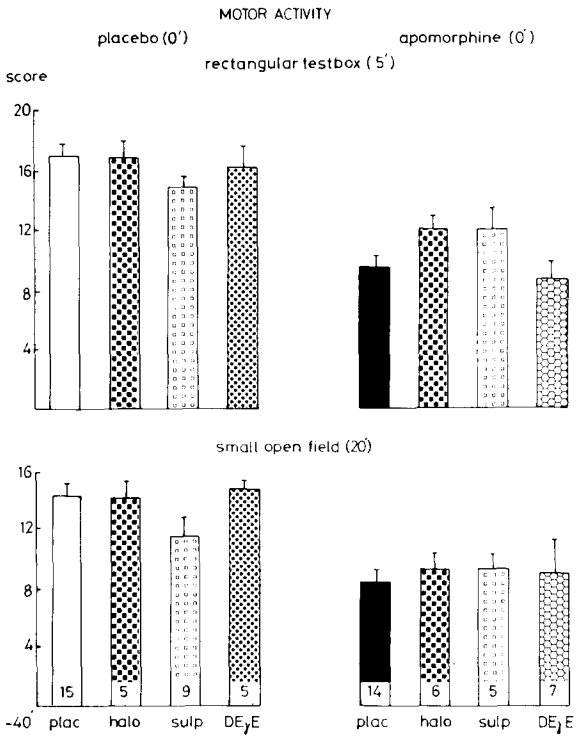


Fig. 4. The influence of pretreatment of the nucleus caudatus with DEγE and neuroleptics (haloperidol, sulpiride) on the apomorphine-induced hypoactivity when apomorphine was given s.c. Motor activity was measured for 3 min at 5 min (rectangular test box) and 20 min (small open field) after injection with placebo (saline) or apomorphine (62.5 μg/kg). Animals were pretreated in the nucleus caudatus with placebo (plac) (1 μl saline) or with haloperidol (halo, 10 pg), sulpiride (sulp, 10 pg) or DEγE (10 ng) 40 min before the s.c. injection of placebo or apomorphine. The mean score for motor activity per treatment group is presented. Vertical bars indicate S.E.M. The number of animals per group is depicted in the columns. ANOVA: placebo vs. haloperidol vs. sulpiride vs. DEγE $P > 0.05$; placebo vs. apomorphine $P < 0.001$.

3.3. Systemically injected apomorphine and pretreatment of the nucleus caudatus

This experiment concerned the effect of pretreatment of the nucleus caudatus with DEγE or neuroleptics on the behavioural effects of apomorphine. The data (fig. 4) show that injections of DEγE (10 ng), haloperidol (10 pg) or sulpiride (10 pg) into the nucleus caudatus could not prevent the effect of s.c. administered apomorphine. This was observed in both test cages (ANOVA: $F(3,28) = 2.7$ and $F(3,28) = 0.2$ with respect to the rectangular test box and small open field respectively). The pretreatments themselves had no effect on motor activity (ANOVA: $F(3,30) = 1.0$ and $F(3,30) = 1.6$ for the rectangular test box and small open field respectively).

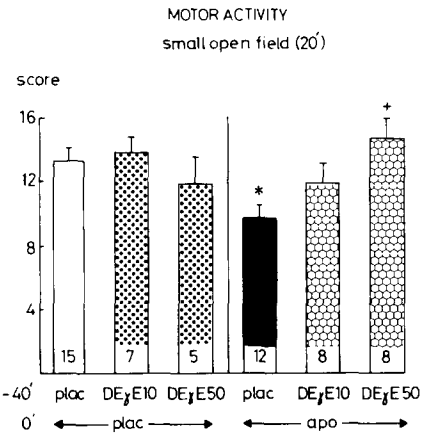


Fig. 5. The influence of s.c. pretreatment with DEγE on the apomorphine-induced hypoactivity when apomorphine was injected into the nucleus accumbens. Motor activity was measured for 3 min, at 20 min (small open field) after injection with placebo (1 μl saline) or apomorphine (10 ng). Animals were pretreated s.c. with placebo (plac) or with DEγE (10 μg or 50 μg per animal) 40 min before the local injection of placebo or apomorphine into the nucleus accumbens. The mean score for motor activity per treatment group is presented. Vertical bars indicate S.E.M. The number of animals per group is shown in the columns. Different from placebo, placebo-treated rats (* $P < 0.05$). Different from placebo, apomorphine-treated rats (+ $P < 0.05$).

3.4. Injection of apomorphine into the nucleus accumbens after systemic pretreatment with DEγE

This experiment concerned the effect of s.c. pretreatment with DEγE on the effects elicited by a local injection of apomorphine into the nucleus accumbens. An injection of apomorphine (10 ng) into the nucleus accumbens decreased motor activity in the small open field (fig. 5). This decrease was completely attenuated in rats pretreated with 50 μg DEγE s.c. A dose of 10 μg DEγE was less effective in this respect (ANOVA: $F(2,25) = 5.8$ ($P < 0.01$)). Neither of the 2 doses of DEγE changed motor activity in placebo-treated rats (ANOVA: $F(2,24) = 0.9$).

4. Discussion

The data confirm previous findings that small doses of apomorphine, injected s.c. or into the nucleus accumbens of rats, decreased motor activity of rats tested in a novel test cage. The present study dealt with the question of which brain structure is involved in this effect of apomorphine on motor activity and exploratory behaviour. When apomorphine is given s.c. It can decrease motor activity as a result of an interaction with any brain structure. However, the data show that the effect of apomorphine was completely blocked by pretreatment of the nucleus accumbens with the DA antagonists haloperidol and sulpiride. The neuropeptide DEγE acts like these DA antagonists in that it also blocks the apomorphine-induced effect when the peptide is applied into the nucleus accumbens. The results further demonstrate that the effect of low doses of apomorphine given s.c. was not affected by the injection of DEγE or DA antagonists into the nucleus caudatus. This suggests that a small dose of apomorphine exerts its effect on motor activity via dopaminergic mechanisms in the mesolimbic system and not in the nigrostriatal system.

The nucleus accumbens has been receiving increased attention, partly because of evidence implicating the mesolimbic dopamine projections to the nucleus accumbens in the control of motor activity (Pijnenburg et al., 1976; Kelly et al., 1975;

Costall and Naylor, 1975). Injections of dopamine, L-DOPA, amphetamine or apomorphine into the nucleus accumbens stimulate locomotor activity in laboratory animals (Andén and Jackson, 1975; Costall et al., 1976; 1980; Pijnenburg et al., 1976).

A number of additional putative neurotransmitters in the nucleus accumbens could also contribute to the control of motor activity. GABAergic receptors in the nucleus accumbens also have been implicated in this respect (Jones et al., 1981). Moreover, the noradrenergic system seems involved in the apomorphine-induced hypoactivity (Sumners et al., 1981).

As far as the effects of dopamine agonists on motor activity and stereotypy are concerned, previous studies have indicated that relatively high doses of apomorphine (10 μg) injected into the nucleus caudatus induced stereotyped sniffing but did not change the motor activity of the animals, whereas similar injections into the nucleus accumbens resulted in hyperactivity but did not change sniffing behaviour (Van Ree et al., 1983). This agrees well with the purported role of the nigrostriatal and mesolimbic dopaminergic systems in the apomorphine-induced stereotypy and hyperactivity respectively (Kelly et al., 1975; Kelly and Iversen, 1976; Pijnenburg et al., 1975; Costall et al., 1977). The stimulant effect of a high dose of apomorphine is attributed to activation of postsynaptic DA receptors in the CNS (Andén et al., 1967). On the contrary, low doses of apomorphine (1-10 ng) injected into the nucleus accumbens decrease motor activity and exploratory behaviour. This depressant effect has been attributed to the preferential stimulation of DA autoreceptors (Carlsson, 1975) resulting in decreased firing of DA neurons (Aghajanian and Bunney, 1973), inhibition of DA synthesis (Strömbom, 1976; Walters and Roth, 1976) and reduction of DA release in vivo (Zetterström and Ungerstedt, 1984) or in vitro (Starke et al., 1978; Westfall et al., 1976).

According to these supposed mechanisms the present data suggest that the presynaptic DA receptors involved in the hypomotility elicited by low doses of apomorphine are localized in the nucleus accumbens. Interestingly, these receptor systems are directly or indirectly blocked by the classic neuroleptic haloperidol, the atypical neuroleptic sulpiride and the presumably antipsychotic peptide DEγE. Thus, this blocking effect might be relevant for the antipsychotic action of these substances. The data further show that DEγE is effective following systemic treatment, an effect which is relevant for the possible clinical use of this neuropeptide in patients suffering from schizophrenic psychoses (Van Ree et al., 1986).

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