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NON-OPIATE β-ENDORPHIN FRAGMENTS AND DOPAMINE—I THE NEUROLEPTIC-LIKE γ-ENDORPHIN FRAGMENTS INTERFERE WITH THE BEHAVIOURAL EFFECTS ELICITED BY SMALL DOSES OF APOMORPHINE

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Summary—In rats, the β- endorphin fragment, 6–17 (des-enkephalin-γ-endorphin, DEγE), dose-dependently antagonized the reduction of the rate of locomotion and rearing induced by small doses of apomorphine. Structure–activity studies revealed that the active moiety of γ-endorphin fragments with respect to counteracting apomorphine-induced behavioural changes resides in the fragment 6–17. The influence of DEγE appeared to be specific for dopamine systems mediating apomorphine-induced hypomotility, since DEγE hardly affected apomorphine-induced stereotypy and amphetamine-induced behavioural changes. These data suggest that DEγE acts as a dopamine antagonist selectively, on those dopamine receptor systems which are stimulated by small doses of apomorphine and which may be located presynaptically.

In contrast to acute treatment, administration of DEγE for 4 days resulted in an enhancement of apomorphine-induced hypomotility. Thus, the receptor systems involved in these effects of apomorphine may become supersensitive upon (sub)chronic treatment with DEγE. The significance of the present findings are discussed in relation to the neuroleptic-like and antipsychotic action of γ-type endorphins.

Des-Tyr^1-γ-endorphin (DTγE, β-endorphin 2–17), a fragment of β-endorphin (β-E) without morphine-like activity, was found to induce neuroleptic-like effects in rats (De Wied, Kovacs, Bohus, Van Ree and Greven, 1978) and to relieve psychotic symptoms of a number of schizophrenic patients (Verhoeven, Van Praag, Van Ree and De Wied, 1979). Evidence has been presented that dopamine (DA) in brain plays an important role in the symptomatology of schizophrenic patients (Matthysse, 1974; Meltzer and Stahl, 1976; Van Praag, 1977; Van Kammen, 1979). In fact, DA activity may be increased in the brain of schizophrenics. Accordingly, most of the currently used neuroleptic drugs block DA receptors (Niemegeers and Janssen, 1979). These data stimulated research on the influence of DTγE on DA systems in brain (Versteeg, De Kloet and De Wied, 1979; Van Ree, Witter and Leysen, 1978; Van Ree, Bohus and De Wied, 1980a; Dorsa, Van Ree and De Wied, 1979; Pedigo, Ling, Reisine and Yamamura, 1979a; Pedigo, Schallert, Overstreet, Ling, Ragan, Reisine and Yamamura, 1979b; Van Ree and Otte, 1980).

Recently, self-inhibiting DA receptor systems (presynaptic DA receptors, and/or DA autoreceptors) have been brought into the discussion of the role of DA in schizophrenia. There is evidence that treatment with small doses of apomorphine decreases DA activity in rodents and attenuates psychotic symptoms of schizophrenic patients, probably as a result of preferentially stimulating self-inhibitory DA receptors (Di Chiara, Porceddu, Vargiu, Argiolas and Gessa, 1976; Di Chiara, Corsini, Mereu, Tissari and Gessa, 1978; Smith, Tamminga and Davis, 1977; Corsini, Del Zempo, Manconi, Piccardi, Onali and Mangoni, 1977; Tamminga, Schaffer, Smith and Davis, 1978). Thus, it was of interest to investigate the interaction between the neuroleptic-like γ-endorphin fragments and small doses of apomorphine. For these studies des-enkephalin-γ-endorphin (DEγE, β-E 6–17) was selected, being one of the main metabolites of DTγE (Burbach, Schotman, Verhoef, De Kloet and De Wied, 1980b) and possessing the neuroleptic-like and antipsychotic activity which is comparable to that of DTγE (De Wied, Van Ree and Greven, 1980; Van Ree, De Wied, Verhoeven and Van Praag, 1980b). It was found that the behavioural effects of small doses of apomorphine were attenuated by acute treatment with DEγE and enhanced following subchronic treatment with this neuropeptide.

Key words: γ-type endorphins, locomotor activity, des-enkephalin-γ-endorphin, stereotypy, apomorphine, amphetamine, neuroleptic-like action, dopamine.

METHODS

Animals and test conditions

Male Wistar rats weighing 130–140 g were used.

The animals were housed under controlled conditions and maintained on a 14:10 light–dark cycle (light on between 5.00 a.m. and 7.00 p.m.) with free access to food and water. Experiments were carried out between 9.00 a.m. and 2.00 p.m. in a sound-attenuated room. Drugs and peptides were administered subcutaneously in the neck of the animals, except for amphetamine which was given intraperitoneally. Each animal was used only once.

Two different test conditions were used to observe the behaviour of the animals: (A) Rectangular perspex observation cages (bottom 20 × 6.5 cm, height 32 cm) and the behaviour was observed for 3 or 4 min as indicated. Locomotor activity and rearing were measured by counting the number of crossings over the midline of the floor and the number of rearings on the hindlegs against the wall of the cage, respectively. In some experiments the duration of stereotyped sniffing and/or licking was measured in sec. (B) Circular perspex test cages (diameter 19.5 cm, height 28.5 cm), the bottom of which was divided into 4 equal sections. In this testbox the following behavioural elements were observed for 5 min: locomotor activity (number of sections explored at least with the forelegs), rearing (facing the wall or in the middle) and stereotypy (total duration of stereotyped sniffing).

DEγE and small doses of apomorphine

Groups of animals ($n = 8$–12) were treated with placebo (0.5 ml saline) or different doses of DEγE (1, 10 or 50 μg) and, after 1 hr, were injected with placebo (0.2 ml saline) or graded doses of apomorphine (1, 5, 25, 125 or 625 μg/kg). Five minutes later, the rats were placed in the rectangular testbox (A) and the behaviour of the rats was observed for 3 min.

Structure–activity relationships

Groups of animals (at least 6 rats per group) were treated with placebo (0.5 ml saline) or 50 μg of different γ-endorphin fragments and, after 1 hr, were injected with placebo (0.2 ml saline) or apomorphine (25 or 125 μg/kg). Five minutes after the last injection, the rats were tested in the testbox (A) for 3 min.

γ-Type endorphins and apomorphine-induced stereotypy

Groups of animals ($n = 10$–17) were injected with placebo (0.5 ml saline) or 50 μg of DEγE or DTγE and, after 1 hr were, treated with placebo (0.2 ml saline) or apomorphine (250 μg/kg). After 20 min, the rats were placed in the circular testbox (B) and the behaviour of the animals was observed for 5 min.

DEγE and amphetamine

Groups of animals ($n = 8$) were subcutaneously treated with placebo (0.5 ml saline) or DEγE (50 μg) and, after 1 hr, were injected intraperitoneally with graded doses of amphetamine (0.08, 0.4, 0.9, 2, 4 or 10 mg/kg). One hour after the last injection, the rats were tested in the rectangular testbox (A) and the behaviour of the rats was scored for 4 min.

Subchronic treatment with DEγE

Animals were injected twice daily (9.00 a.m. and 5.00 p.m.) with placebo (0.5 ml saline) or DEγE (10 μg) for 4 days. Twenty to twenty two hours after the last injection, groups of animals ($n = 18$) were treated with placebo (0.2 ml saline) or apomorphine (25 or 125 μg/kg) and after 5 min the behaviour of the rats was observed for 3 min using testbox (A).

Analysis of the data

Groups mean ± SEM were calculated. For statistical analysis, two tailed Student's *t*-tests were used. The data of the experiments concerning structure–activity relationships were first analysed by one way analysis of variance (ANOVA) testing.

Drugs and peptides

Apomorphine (Apomorphine HCl) and amphetamine (Dexamphetamine sulfate) were obtained from O.P.G., Utrecht, The Netherlands. The following fragments of β-endorphin (βE) were used: des-Tyr^1-γ-endorphin (DTγE, βE 2–17); βE 5–17; des-enkephalin-γ-endorphin (DEγE, βE 6–17); βE 7–17; βE 8–17; βE 9–17 and βE 10–17. They were prepared by the classical approach of fragment condensation and analysed, among others, by high performance liquid chromatography. The purity of the peptides appeared to be 95–99%. They were kindly donated by Dr H. M. Greven, Organon International B.V., Oss, The Netherlands.

RESULTS

DEγE and small doses of apomorphine

Apomorphine dose-dependently decreased the rate of locomotion (Fig. 1) and the incidence of rearing (Fig. 2), as assessed at 5 min after its subcutaneous injection in a small testbox. There was a significant effect even after the smallest dose (1 μg/kg) of apomorphine. Animals pretreated with 50 μg of DEγE and injected with placebo just prior to testing showed a slight, albeit significant, decrease in the rate of locomotion (Fig. 1). The decrease of locomotor activity induced by small doses of apomorphine was markedly attenuated in rats pretreated with DEγE (Fig. 1). A similar effect of DEγE was observed on the apomorphine-induced decrease of rearing, although the effect of DEγE was somewhat less than that on apomorphine-induced decrease of locomotion. This is probably due to the individual variation in the frequency of rearing. The influence of DEγE appeared to be dose-related and competitive (Fig. 2).

Structure–activity relationships

None of the peptides tested significantly affected the rate of locomotion and rearing of rats injected with placebo just prior to testing (ANOVA: $F = 0.67$ (7,65) and $F = 0.59$ (7,65) respectively). The peptide DEγE significantly attenuated the decrease of loco-

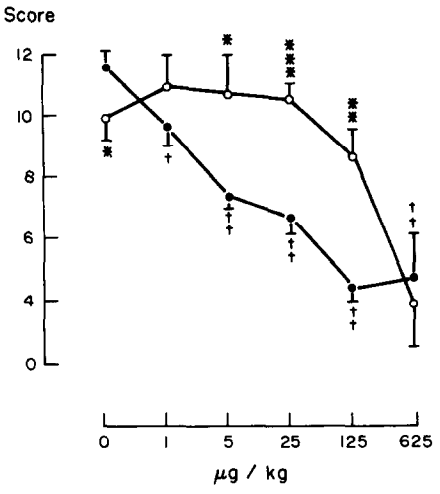

Fig. 1. The influence of pretreatment with DEγE (des-enkephalin-γ-endorphin) on the apomorphine-induced decrease of locomotor activity of rats. Locomotion was measured for 3 min, beginning 5 min after subcutaneous injection with placebo or graded doses of apomorphine HCl. Animals were injected subcutaneously with placebo (●——●) or 50 μg DEγE (○——○) 1 hr before the apomorphine. The mean locomotion score of 8 animals per treatment group was plotted against the dose of apomorphine. Vertical bars indicate SEM. *Different from placebo-pretreated rats (*$P < 0.02$, **$P < 0.01$, ***$P < 0.001$). †Different from rats not treated with apomorphine (placebo–placebo controls) (†$P < 0.02$, ††$P < 0.001$).

motion and rearing elicited by 25 and 125 μg/kg apomorphine (Fig. 3). In general, the peptides DTγE and βE 5–17 mimicked the effect of DEγE. The effect of DTγE did not reach statistical significance in rats treated with the smaller dose of apomorphine. This may have been due to a slight decrease in locomotion and rearing induced by DTγE in the placebo controls, since no effect of apomorphine was found in DTγE pretreated animals (placebo vs apomorphine 25 μg/kg). The analogue βE 5–17 appeared to be somewhat less active than DTγE and DEγE. The peptide βE 7–17 had a slight, but not statistically significant attenuating effect in rats treated with 125 μg/kg apomorphine, while the peptides βE 8–17, βE 9-17 and βE 10–17 were virtually inactive.

γ-Type endorphins and apomorphine-induced stereotypy

Rats treated with 250 μg/kg apomorphine and tested 20 min later in the circular testbox displayed a slight, although not statistically significant, increase of locomotion and decrease of rearing as compared to saline-treated controls (Fig. 4). For about half of the time of testing the apomorphine-treated rats showed stereotyped sniffing. Pretreatment with DEγE or DTγE did not influence these apomorphine-induced behavioural changes.

DEγE and amphetamine

Amphetamine increased the rate of locomotion and rearing at doses of 0.9–4 mg/kg, while a marked decrease of these behavioural elements was observed at the 10 mg/kg dose (Fig. 5). Some stereotyped sniffing was present in animals treated with 0.9 mg/kg. This behaviour was displayed during nearly the whole test time following injection with 4 mg/kg. Pretreatment with DEγE hardly affected the amphetamine-induced behavioural changes. Neither the increase in locomotion and rearing nor the stereotyped sniffing was at-

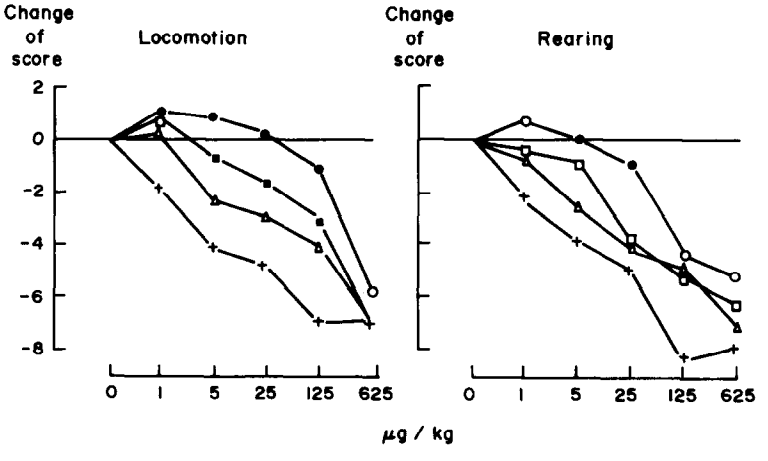

Fig. 2. The influence of pretreatment with DEγE (des-enkephalin-γ-endorphin) on the apomorphine-induced decrease of locomotor activity and rearing of rats. Locomotion and rearing was measured for 3 min, beginning 5 min after subcutaneous treatment with placebo or graded doses of apomorphine–HCl. Animals were injected subcutaneously with placebo (+——+) or 1 μg (△——△), 10 μg (□——□) or 50 μg (○——○) DEγE 1 hr before apomorphine. The change in locomotion and rearing score of 8–12 animals per treatment group was plotted against the dose of apomorphine. All doses of apomorphine induced in placebo pretreated rats a statistically significant decrease of locomotion and rearing as compared to placebo controls ($P < 0.05$). Filled symbols indicate a statistically significant attenuation of the apomorphine effect ($P < 0.05$).

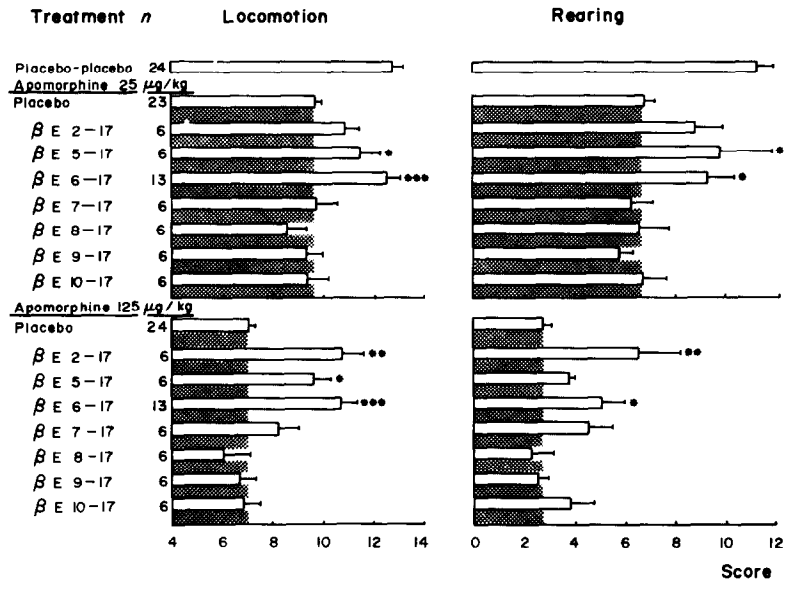

Fig. 3. The influence of several γ-endorphin (β-endorphin (βE) 1–17) fragments on apomorphine induced decrease of locomotor activity and rearing of rats. Locomotion and rearing was measured for 3 min, beginning 5 min after subcutaneous treatment with placebo or apomorphine–HCl (25 or 125 µg/kg). Animals were injected subcutaneously with placebo or 50 µg of peptide 1 hr before apomorphine. ANOVA testing revealed for rats treated with 25 µg/kg apomorphine F:4.66 (7,64) and 1.91 (7,64) for locomotion ($P < 0.01$) and rearing ($P < 0.1$) respectively and for rats treated with 125 µg/kg F: 6.10 (7,65) and 2.73 (7,65) for locomotion ($P < 0.01$) and rearing ($P < 0.05$) respectively. n = Number of animals. *Different from rats pretreated with placebo (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

tenuated or enhanced by DEγE. Only at 4 mg/kg of amphetamine was a significant decrease of locomotion found in rats pretreated with DEγE as compared to placebo-injected controls.

Subchronic treatment with DEγE

Apomorphine dose-dependently decreased locomotion and rearing in rats treated with placebo twice daily for 4 days (Fig. 6). Rats treated daily with DEγE, and injected with placebo just prior to testing, disdisplayed a similar amount of locomotor activity and rearing as placebo controls. However, the DEγE-pretreated rats appeared to be more sensitive to apomorphine, as compared to placebo-pretreated rats, in that the apomorphine-induced reduction of locomotion was enhanced. A similar tendency was observed with respect to the apomorphine-induced decrease of rearing, although the differences did not reach statistical significance.

DISCUSSION

The data from this series of experiments show that acute treatment with DEγE antagonized the behavioural effects observed shortly after the injection of small doses of apomorphine, i.e. decrease of locomo-

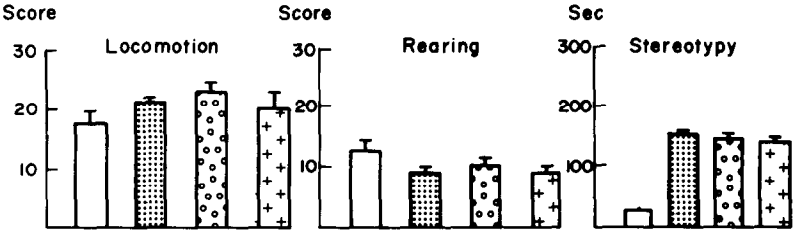

Fig. 4. The influence of γ-endorphin fragments on apomorphine-induced behavioural changes as assessed in a small open field. The rate of locomotion and rearing and the duration of stereotyped sniffing was measured for 5 min, beginning 20 min after subcutaneous treatment with placebo or apomorphine–HCl (250 µg/kg). Groups of animals were injected subcutaneously with placebo or 50 µg of DTγE (des-Tyr^1-γ-endorphin) or DEγE (des-enkephalin-γ-endorphin) 1 hr before apomorphine. Results are given as mean ± SEM (vertical bars). Treatments: □ placebo, placebo (n = 11) ▦ placebo, apomorphine (n = 17) ▣ DTγE, apomorphine (n = 17) ⊞ DEγE, apomorphine (n = 10).

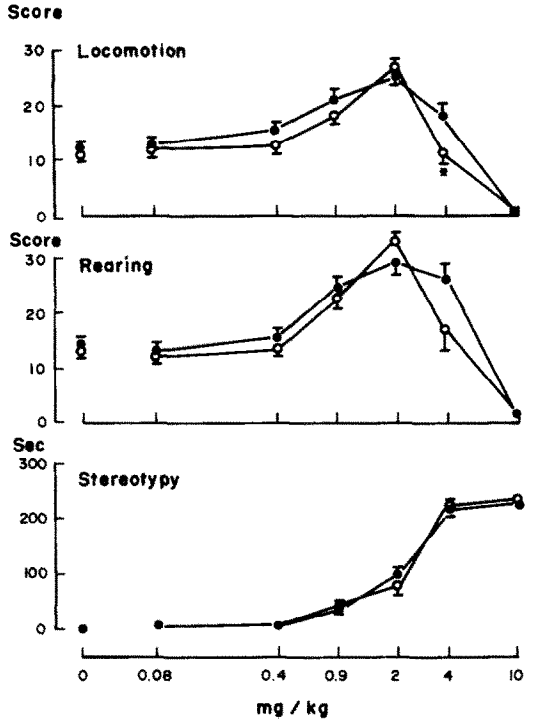

Fig. 5. Effect of pretreatment with DEγE (des-enkephalin-γ-endorphin) on the amphetamine-induced stereotypy and changes in locomotion and rearing. The rats were tested for 4 min, 1 hr after intraperitoneal injection with placebo or graded doses of amphetamine. Animals were injected subcutaneously with placebo (●——●) or 50 μg DEγE (○——○) 1 hr before amphetamine. The mean locomotion and rearing score and the duration of stereotyped sniffing and/or licking (sec) of 8 animals per treatment group was plotted vs the dose of amphetamine. Vertical bars indicate SEM. *Different from placebo pretreated rats ($P < 0.05$).

tion and rearing. The hypomotility induced in rodents by apomorphine has been suggested to be mediated by self-inhibitory DA receptors (Di Chiara *et al.*, 1976, 1978; Strömbom, 1977; Corsini *et al.*, 1977; Tamminga *et al.*, 1978). This suggestion is based on the fact that DA antagonists and neuroleptic drugs such as haloperidol, pimozide and sulpiride prevent the apomorphine-induced hypomotility and decrease of brain DOPAC levels, most probably as a result of a decreased DA neuronal output. This effect is presumably mediated by an agonist effect of apomorphine on self-inhibitory DA receptors. Also, electrophysiological studies suggest that these receptors are much more sensitive to apomorphine than are postsynaptic DA receptors and that activation of the self-inhibitory receptors leads to a reduction in firing rate of DA neurones (Skirboll, Grace and Bunney, 1979). Thus, the findings described here suggest that DEγE may interact directly or indirectly with apomorphine-sensitive self-inhibitory DA receptor systems.

The influence of DEγE is specific for γ-type endorphins. Structure–activity relationship studies showed that DTγE and βE 5–17 induced a similar effect to that observed with DEγE. Removal of aminoacids from the N terminal end of DEγE led to peptides with less, if any, activity in this respect. The same holds true for the C-terminal end of DEγE, since removal of leucine from DEγE and DTγE, which yields α-type endorphin peptides, also destroyed the action of γ-type endorphins with respect to their inhibitory effect on apomorphine-induced hypomotility (Van Ree, 1982). Thus, DEγE appears to be the shortest sequence of γ-endorphin which counteracts the apomorphine-induced behavioural effects. This conclusion agrees very well with previous findings showing that DEγE is the active moiety of γ-endorphin with respect to neuroleptic-like activity as assessed by the extinction of pole-jumping avoidance behaviour and with the peptide-induced grasping response (De Wied *et al.*, 1980). Since the apomorphine-induced hypomotility is attenuated by low doses of neuroleptic drugs, the present data further substantiate the neuroleptic-like activity of γ-type endorphins.

The interaction of γ-type endorphins with brain DA systems appears to be quite complex. Intracerebroventricular administration of DTγE increased the α-methyl-*p*-tyrosine-induced disappearance of DA in a number of discrete regions of the rat brain, particularly in terminal regions of the intradiencephalic DA systems (Versteeg *et al.*, 1979; Versteeg, Kovacs, Bohus, De Kloet and De Wied, 1982). Subcutaneously

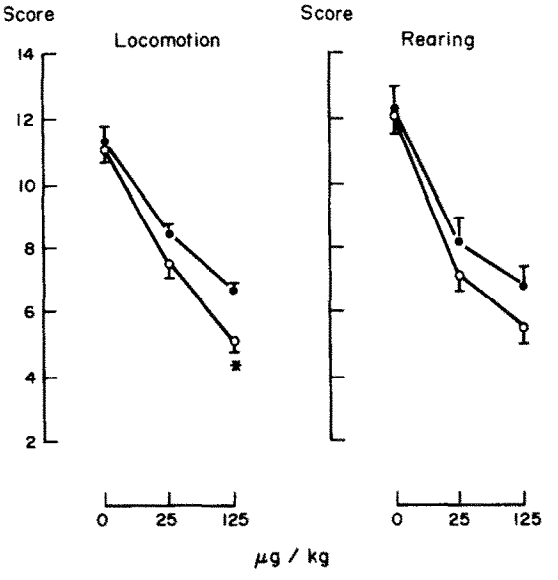

Fig. 6. Effect of subchronic treatment with DEγE (des-enkephalin-γ-endorphin) on the apomorphine-induced decrease of locomotor activity and rearing of rats. Rats were treated subcutaneously with 10 μg DEγE (○——○) or placebo (●——●) twice daily for 4 days. On the 5th day locomotion and rearing was measured for 3 min, beginning 5 min after the subutaneous injection of placebo (0 μg/kg) or apomorphine–HCl (25 or 125 μg/kg). The mean locomotion and rearing score of 18 animals per treatment group was plotted vs the dose of apomorphine. Vertical bars indicate SEM. *Different from placebo-pretreated rats ($P < 0.001$).

injected DTγE decreased the electrical self-stimulating behaviour elicited from electrodes located in the ventral tegmental and the nucleus accumbens area where DA cell bodies and terminals of the mesolimbic DA pathway are present, respectively (Dorsa *et al.*, 1979; Van Ree and Otte, 1980). The peptide DTγE mimics the action of DA antagonists in reducing ACTH-induced excessive grooming when injected into the nucleus accumbens or the nucleus caudatus (Gispen, Ormond, Ten Haaf and De Wied, 1980). Although DTγE did not displace [^{3}H]haloperidol, [^{3}H]spiperone and [^{3}H]apomorphine from their stereospecific binding sites in rat brain membrane preparations *in vitro* (Van Ree *et al.*, 1978, 1980a; Pedigo *et al.*, 1979a), it did attenuate *in vivo* binding of [^{3}H]spiperone (Pedigo *et al.*, 1979b). Thus, DTγE may affect DA receptor systems in an indirect manner or after conversion to an active metabolite, e.g. DEγE (Burbach *et al.*, 1980b; De Wied *et al.*, 1980 and this paper). Moreover, γ-type endorphins may interact with one specific class of the multiple DA receptor system. Different subclassifications of DA receptors have been proposed, e.g. excitation-mediating (DAe) vs inhibition-mediating (DAi) receptors (for Ref. see Cools and Van Rossum, 1980). D1 vs D2 receptors (for Ref. see Kebabian and Calne, 1979) (recently extended to D3 and D4 receptors, see Seeman, 1980) and postsynaptically located DA receptors vs receptors located at presynaptic membranes of the DA synapses and at membranes of the DA cell bodies [self-inhibitory DA receptors (Carlsson, 1975)]. Classical neuroleptic drugs, e.g. haloperidol may be antagonists of most, if not all, DA receptors, although their potency in blocking the actions of DA may vary among the various DA systems. The neuroleptic-like peptide DEγE may be more discriminating in this respect. The present data show that DEγE attenuated apomorphine-induced hypomotility, but not the stereotypy induced by either apomorphine or amphetamine. In addition, DEγE did not affect amphetamine-induced hyperactivity. Apomorphine has been reported to display agonist activity on DAe receptors and to have little, if any, activity on DAi receptors and to activate presynaptically located DA receptors at much lower concentrations than for those located postsynaptically. The hypomotility elicited by apomorphine may thus be mediated by self-inhibiting DA receptors, while apomorphine-induced stereotypy is suggested to be linked to DAe and/or postsynaptically located DA receptors. The amphetamine-induced hyperactivity and stereotypy may be due to release of DA and subsequent activation of postsynaptically located DA receptors (DAe/D1 receptors). According to the classification of Seeman (1980), the hypoactivity induced by small doses of apomorphine may be mediated by D3 or D4 receptors, since these sites bind dopamine agonists at nanomolar concentrations while D1 and D2 receptors are sensitive to micromolar concentrations of these substances. Thus, it might be proposed that DEγE interferes selectively with receptor systems sensitive to small doses of DA agonists, presumably located presynaptically, although a postsynaptic location of these receptors cannot be excluded as yet. Acute treatment with DEγE mimics the action of neuroleptics in inhibiting the actions of DA on these receptor systems. Also other data have been interpreted as indicating that γ-type endorphins interfere with presynaptic DA systems (Nickolson and Berendsen, 1980).

(Sub)chronic treatment with DA antagonists induces supersensitivity for DA receptor systems mediating apomorphine- and amphetamine-induced stereotypy (post-synaptically located) and those mediating apomorphine-induced hypoactivity (Muller and Seeman, 1978; Verimer, Goodale, Long and Flynn, 1980). These changes in sensitivity might explain the present findings that DEγE acts as a DA antagonist after acute treatment and that sensitivity to apomorphine is increased after subchronic treatment with this peptide. This increased sensitivity may have deen due to an altered sensitivity of the DA system rather than to the presence of the peptide itself, since the increased sensitivity persisted at least 20 hr. Thus, also with respect to supersensitivity, DEγE mimics the action of neuroleptics on the receptor system mediating apomorphine-induced hypomotility. The exact nature of the interaction of DEγE with these receptor systems is not known. Several possibilities can be offered to explain this interaction: an affinity of DEγE for the DA receptor, an alteration in the translation of receptor activation, modulation of the receptor structure, a change in the number of receptor molecules etc. The research on this subject may be hampered by the proposed interference of DEγE with a selective number of DA receptor systems.

Since DEγE and closely related peptides may be continuously present under normal conditions of brain homeostasis (Verhoef, Loeber, Burbach, Gispen, Witter and De Wied, 1980; Burbach, Loeber, Verhoef, Wiegant, De Kloet and De Wied, 1980a), and subchronic treatment with DEγE induced supersensitivity of receptor systems mediating apomorphine-induced hypomotility, it may be postulated that a chronic deficiency of this peptide could lead to a state of subsensitivity of these DA receptor systems. According to the hypothesis that psychosis of the schizophrenic type is due to a relative deficiency of γ-type endorphins (De Wied, 1978), these DA receptor systems in the schizophrenic brain may be subsensitive, resulting in a sustained increase in DA activity which may be corrected by chronic treatment with γ-type endorphins as well as neuroleptic drugs. This postulate may serve to link the 'DTγE and related peptides' hypothesis with the 'dopamine' hypothesis of schizophrenia. However, more studies are needed to verify this postulate.

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