γ -ENDORPHIN AND N ^{α} -ACETYL- γ -ENDORPHIN INTERFERE WITH DISTINCT DOPAMINERGIC
SYSTEMS IN THE NUCLEUS ACCUMBENS VIA OPIOID AND NON-OPIOID MECHANISMS

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Summary

Low doses (10 ng) of the dopamine agonist apomorphine induced hypolocomotion when injected into the nucleus accumbens of rats. This behavioral response was antagonized by local treatment with either the opioid peptide γ -endorphin (γ E) or the non-opioid peptide N ^{α} -acetyl- γ -endorphin (Ac γ E) in a dose of 100 pg. High doses of apomorphine (10 μ g) or amphetamine (2 μ g) injected into the nucleus accumbens resulted in hyperlocomotion. This response was blocked by pretreatment with γ E but not with Ac γ E. This effect of γ E could be prevented by local treatment with naloxone. Neither peptides interfered with the apomorphine-induced stereotyped sniffing when the substances were injected into the nucleus caudatus. It is concluded that γ E and Ac γ E differentially interact with distinct dopaminergic systems in the nucleus accumbens of the rat brain via an opioid and a non-opioid mechanism, suggesting that the peptide fragments originating from pro-opiomelanocortin may be specifically implicated in the control of dopaminergic activity in this brain area.

The opioid peptide γ -endorphin (γ E) produces behavioral effects in rats which resemble those of neuroleptics in certain aspects (1-3). This neuroleptic-like action can also be elicited by fragments of γ E i.c. des-Tyr¹- γ -endorphin (DT γ E) and des enkephalin- γ -endorphin (DE γ E), that are devoid of opiate activity (1,4-6). Thus, the opiate and neuroleptic-like action of γ E may be mediated by distinct brain mechanisms. Research in this respect is especially focussed on brain dopamine (6-9), since neuroleptic drugs are potent blockers of dopaminergic activity (10), and opioid peptides have been implicated in the control of certain dopaminergic systems in the brain (11). Previously, we have reported that following subcutaneous administration DT γ E and DE γ E antagonized the hypolocomotion induced by low doses of the dopamine agonist apomorphine, while these peptides did not interfere with the hyperlocomotion and stereotyped behavior elicited by high doses of apomorphine (6,12). Subsequently, it was found that low doses of apomorphine injected directly into the nucleus accumbens resulted in hypolocomotion (13). This behavioral response was antagonized by pretreatment with DT γ E and DE γ E (7). High doses of apomorphine injected into the nucleus accumbens produced hyperlocomotion. This response was not affected by pretreatment with DE γ E (14). From these studies it was concluded that γ -type endorphins interfere with certain, but not all, dopaminergic systems in the nucleus accumbens.

Recently, N ^{α} -acetyl- γ -endorphin (Ac γ E) has been isolated from pituitary

and brain tissue and shown to possess little, if any opiate activity (15). In the present series of experiments we have further explored the interaction of γ -type endorphins, especially γ E and Ac γ E, with the dopaminergic systems present in the nucleus accumbens. As testprocedure we used the behavioral effects elicited by injection of the dopamine agonist apomorphine and in some experiments amphetamine. The data show that γ E and Ac γ E differentially interact with distinct dopaminergic systems in the nucleus accumbens via an opioid and a non-opioid mechanism.

Materials and Methods

Animals and surgery

Male Wistar rats of an inbred strain (TNO, Zeist, The Netherlands) weighing between 140-165 g were used. They were kept under standard conditions (room temperature $22 \pm 1^\circ\text{C}$, light on from 7.00 a.m. until 7.00 p.m.), were housed in groups of 5 and received food and water ad libitum. Rats were anesthetized with Hypnorm[®], secured in a stereotaxic instrument and stainless steel guide cannulae (0.6 mm outer dia, 0.3 mm inner dia) were implanted bilaterally, as described before (12,13). For implantation into the nucleus accumbens area the coordinates were 2.6 mm anterior to bregma, 2.7 mm lateral to the midline, 6.1 mm below the dura at the point of penetration and the cannulae were inserted at an angle of 12° . For implantation into the nucleus caudatus the coordinates were 2.0 mm anterior to bregma, 2.5 mm lateral to the midline, and 6.0 mm below the skull, according to Pellegrino and Cushman (16) except that the level of the upper incisor bar was at the level of the interaural line. Operated animals were housed in single cages. Behavioral testing was performed at least one week after operation.

Behavioral testing

For determining locomotor activity and sniffing a small open field was used (for details see 6,7,12,13). The bottom of this circular perspex testcage (diameter 19.5 cm, height 28.5 cm) was divided into 4 equal sections. In this small open field locomotor activity (number of sections explored) and the duration (sec) of (stereotyped) sniffing were measured for 3 or 5 min. Rats with cannulae into the nucleus accumbal area were injected twice. They were treated with peptide (100 pg or 10 ng) or placebo and after 40 min with apomorphine (10 ng or 10 μg), amphetamine (2 μg) or placebo. In some experiments a third injection with placebo or naloxone (2 μg) was given 10 min before apomorphine. Twenty min after apomorphine treatment the rats were tested in the small open field for 3 min. The rats used in the experiments with a high dose of apomorphine were habituated to the testcage, by placing them twice in the small open field on the days before testing. Rats with cannulae into the nucleus caudatus were injected with peptide (10 ng) or placebo and after 1 h were treated with apomorphine (10 μg), or placebo. After 20 min the rats were placed in the small open field and the behavior of the animals was observed for 5 min. In general, the rats were tested twice, with an interval of at least one week.

Drugs and injections

The peptides used were γ -endorphin (γ E, H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH) and N ^{α} -acetyl- γ -endorphin (Ac γ E). The peptides were donated by Dr. H.M. Greven, Organon International B.V., Oss, The Netherlands. Apomorphine (Apomorphine HCl) and amphetamine (Dexamphetamine sulphate) were obtained from OPG, Utrecht, The Netherlands. The peptides and drugs were stored dry and dissolved in saline immediately prior to use in a volume of 1 μl . Placebo treated rats received a similar volume of saline.

Intracerebral injections were performed using a Hamilton syringe by inserting a needle into the stainless steel guide cannula.

Histology

After experimentation the injection sites were determined histologically. The rats were sacrificed and the brains fixed in 4% formaline. Serial sections were cut with a thickness of 100 μ m on a cryostat. The sites of injections were inspected microscopically using the atlas of Pellegrino and Cushman (16).

Statistical analysis

The data of experiments were first analyzed using one way ANOVA testing and when the outcome revealed a statistically significant effect ($p < 0.05$) subsequently analyzed with Student's t -tests.

Results

The sites of injections aimed at the nucleus accumbens appeared to be bilaterally in the middle and anterior part of the nucleus accumbens as reported before (13). Those in the nucleus caudatus were present in the middle of the nucleus caudatus (12). Data from rats with cannulae outside the nucleus accumbens and the nucleus caudatus respectively were discarded from further analysis.

Low doses of the dopamine agonist apomorphine (10 ng) injected into the nucleus accumbens resulted in hypolocomotion of the rats (fig. 1), whereas relatively high doses of this drug (10 μ g) elicited hyperlocomotion (fig. 2). Neither low nor high doses of apomorphine changed the duration of sniffing of the rats (Table I). Pretreatment with 100 pg of γ E and Ac γ E antagonized the hypolocomotion, induced by low doses of apomorphine (fig. 1). However, when 10 ng of these peptides was injected differential effects were observed, in that Ac γ E inhibited the apomorphine-induced hypolocomotion, whereas γ E was not effective in this respect (fig. 1). This dose of Ac γ E and γ E induced a slight decrease of locomotion per se (fig. 1). In subsequent experiments the effects of pretreatment with the peptides on the hyperlocomotion induced by relatively high doses of apomorphine (10 μ g) or amphetamine (2 μ g) were determined. It was found that γ E (10 ng) antagonized the hyperlocomotion induced by both drugs, whereas Ac γ E did not significantly interfere with this behavioral response (fig. 2). A lower dose of γ E (100 pg) partly inhibited the amphetamine (2 μ g) induced hyperlocomotion (locomotion scores: placebo, placebo: $16,3 \pm 0.5$; placebo, amphetamine: $23,3 \pm 0.7$; γ E, amphetamine: $19,7 \pm 0.8$, $p < 0.01$ as compared to placebo, amphetamine; $n=6/\text{group}$). The peptides did not interfere with the duration of sniffing in placebo nor in drug treated rats (Table I).

In the next series of experiments the opiate antagonist naloxone was injected 10 min before apomorphine (10 μ g) or amphetamine (2 μ g) administration (Table II). As found before, both apomorphine and amphetamine induced hyperlocomotion that could be antagonized by pretreatment with 10 ng of γ E. Naloxone injection did not affect the response elicited by apomorphine and amphetamine. However, naloxone completely or partly antagonized the γ E effect on the hyperlocomotion response (Table II).

Injection of apomorphine into the nucleus caudatus resulted in an increase in the duration of sniffing (Table III). The sniffing observed following apomorphine was of a stereotyped nature. The locomotor behavior of the rats was not changed by this treatment. Neither γ E nor Ac γ E interfered with the stereo-

FIG. 1

Effect of γ -endorphin (γ E) and N^α-acetyl- γ -endorphin (Ac γ E) on the apomorphine-induced hypolocomotion following injection into the nucleus accumbens. Locomotor activity was assessed in a small, circular open field for 3 min, 20 min after placebo (plac, 1 μ l saline) or apomorphine treatment. Pretreatment with peptides or placebo was performed 60 min before testing. The mean score $\pm$ S.E.M. (vertical bars) of the various treated groups are presented. The number of animals per group are depicted in the columns. Data ANOVA testing: $F = 30,0$ (7,73), $p < 0.01$. * different from placebo, placebo treated rats (* $p < 0.005$; ** $p < 0.001$). + different from placebo, apomorphine treated rats ($p < 0.001$).

typed sniffing response following apomorphine injection into the nucleus caudatus. The peptides did also not change the baseline sniffing behavior (Table II).

Discussion

The present data show that at least two dopaminergic systems are present in the nucleus accumbens, one activated by low doses of apomorphine, resulting in hypolocomotion and the other activated by high doses of apomorphine and probably indirectly by amphetamine, resulting in hyperlocomotion. These two systems can also be distinguished with the neuroleptic drugs haloperidol and sulpiride. Thus, 10 - 30 pg of these neuroleptics injected into the nucleus accumbens antagonized the apomorphine-induced hypolocomotion (7), while similar doses of haloperidol, in contrast to sulpiride, attenuated the hyperlocomotion induced by apomorphine or amphetamine (unpublished data). It has been postulated that the effects of low doses of apomorphine are mediated by presynaptically located dopaminergic receptor systems, which activation results in a decreased dopamine output and consequently in hypolocomotion (for ref. see 6,7,13). Activation of postsynaptically located dopaminergic receptors either directly

FIG. 2

Effect of γ -endorphin (γ E) and N^α-acetyl- γ -endorphin (Ac γ E) on the hyperlocomotion elicited by apomorphine or amphetamine following injections into the nucleus accumbens. Locomotor activity was assessed in a small, circular open field for 3 min, 20 min after placebo (plac, 1 μ l saline), apomorphine or amphetamine treatment. Pretreatment with peptides (10 ng) or placebo was performed 60 min before testing. The mean score $\pm$ S.E.M. (vertical bars) of the various treated groups are presented. The number of animals per group are depicted in the columns. Data ANOVA testing: apomorphine experiment $F = 21,4$ (3,32), $p < 0.01$; amphetamine experiment $F = 17,5$ (3,25), $p < 0.01$.

* different from placebo, placebo treated rats ($p < 0.001$).

+ different from placebo, apomorphine or placebo, amphetamine treated rats ($p < 0.001$).

by high doses of apomorphine or probably indirectly by amphetamine via release of dopamine, would result in hyperlocomotion. Although this concept is evidenced by a number of experimental data, it is still possible that the differential sensitivity to apomorphine belongs to more distinct brain pathways. High doses of apomorphine injected into the nucleus accumbens resulted in hyperlocomotion without affecting sniffing behavior, while similar injections into the nucleus caudatus induced stereotyped sniffing without changing the locomotor activity of the animals. This agrees well with the purported role of the mesolimbic and nigro-striatal dopaminergic systems with projections to the nucleus accumbens and nucleus caudatus respectively in the apomorphine-induced hyperactivity and stereotypy respectively (17-20).

It was found in the present experiments that γ E affects both dopaminergic systems in the nucleus accumbens, in that the hypo- and hyperlocomotion elicited by low respectively high doses of apomorphine were antagonized, whereas the non-opiate-like peptide Ac γ E antagonized selectively the effects of low doses

TABLE I

Sniffing Behavior of Rats Treated with Drugs and Peptides into the Nucleus Accumbens of Rats. Duration of Sniffing (Sec) Was Assessed in a Small, Circular Open Field for 3 Min

Pretreatment Treatment	placebo	γE (10 ng)	AcγE (10 ng)	ANOVA F value
placebo	21.6 ± 1.6 (38)	30.4 ± 1.4 (5)	21.6 ± 3.4 (5)	2,09 (2,43)
apomorphine (10 ng)	17.7 ± 1.2 (22)	19.1 ± 3.0 (9)	24.4 ± 2.9 (5)	1,71 (2,32)
apomorphine (10 μg)	20.8 ± 1.8 (10)	19.2 ± 2.1 (9)	14.7 ± 1.8 (7)	2,35 (2,23)
amphetamine (2 μg)	28.5 ± 1.9 (11)	24.6 ± 0.4 (5)	30.2 ± 2.8 (5)	1,37 (2,18)

Rats were pretreated 60 min and treated 20 min before testing. () number of animals. Amphetamine induced a slight increase in the duration of sniffing in placebo pretreated rats, but this effect was not significant, when compared with simultaneously tested rats treated with placebo.

TABLE II

Influence of Naloxone on the Antagonizing Action of γ-Endorphin (γE) on the Hyperlocomotion Elicited by Injection of Apomorphine or Amphetamine into the Nucleus Accumbens of Rats. Locomotor Activity Was Assessed in a Small, Circular Open Field for 3 Min

Treatment			Locomotor activity scores	
-60'	-30'	-20'	(mean ± S.E.M.)	
A.				
placebo	placebo	placebo	15.5 ± 0.7	(6)
placebo	placebo	apomorphine 10 μg	24.2 ± 0.8	(6)
placebo	naloxone 2 μg	apomorphine 10 μg	23.2 ± 0.9	(5)
γE 10 ng	placebo	apomorphine 10 μg	15.0 ± 1.1	(6)
γE 10 ng	naloxone 2 μg	apomorphine 10 μg	23.9 ± 1.2	(7)
B.				
placebo	placebo	placebo	17.2 ± 0.5	(6)
placebo	placebo	amphetamine 2 μg	27.2 ± 1.4	(6)
placebo	naloxone 2 μg	amphetamine 2 μg	27.8 ± 1.5	(5)
γE 10 ng	placebo	amphetamine 2 μg	16.7 ± 0.9	(6)
γE 10 ng	naloxone 2 μg	amphetamine 2 μg	22.8 ± 1.3	(6)

* differences between the indicated treatment groups (* p < 0.005, ** p < 0.001, Student's t-test). () number of animals. Data ANOVA testing: A: F = 22,2 (4,25), p < 0.01; B: F = 20,6 (4,24), p < 0.01.

TABLE III

Effect of γ -Endorphin (γ E) and N^α-Acetyl- γ -Endorphin (Ac γ E) on the Behavioral Changes Induced by Injection of Apomorphine into the Nucleus Caudatus.

The Behavior of the Rats Was Assessed in a Small, Circular Open Field for 5 Min

Treatment		Locomotor activity (scores $\pm$ S.E.M)	Duration of (stereotyped) sniffing (sec $\pm$ S.E.M)	
-80'	-20'			
placebo	placebo	29.4 $\pm$ 2.8	17.5 $\pm$ 1.9	(12)
γ E (10 ng)	placebo	24.3 $\pm$ 3.7	18.9 $\pm$ 3.2	(7)
Ac γ E (10 ng)	placebo	31.5 $\pm$ 2.6	19.7 $\pm$ 2.2	(6)
placebo	apomorphine (10 μ g)	24.9 $\pm$ 3.4	46.3 $\pm$ 2.2 ⁺	(10)
γ E (10 ng)	apomorphine (10 μ g)	32.3 $\pm$ 4.7	44.5 $\pm$ 2.2 ⁺	(6)
Ac γ E (10 ng)	apomorphine (10 μ g)	34.6 $\pm$ 4.1	52.8 $\pm$ 3.8 ⁺	(8)

() number of animals.

Data ANOVA testing: locomotor activity $F = 1,33$ (5,43); sniffing $F = 38,82$ (5,43). ⁺ $p < 0.001$ as compared to rats injected with placebo instead of apomorphine (Student's t -tests).

of apomorphine. This suggests that the effects of γ E on the apomorphine-induced hyperlocomotion are mediated by the inherent opiate activity of this peptide. Indeed, it was found that the antagonizing action of γ E on the hyperlocomotion induced by apomorphine or amphetamine was at least partly reversed by local treatment with the opiate antagonist naloxone. The opiate system mediating the effects of γ E may be specifically activated by γ E since neither β -endorphin nor met-enkephalin mimicked the action of γ E (unpublished data). Thus, γ E has a dual action on dopaminergic systems in the nucleus accumbens, one of which is mediated by a naloxone sensitive mechanism. This last mentioned action could be responsible for the ineffectiveness of the 10 ng dose of γ E to affect the apomorphine-induced hypolocomotion. The effect of γ E could not be generalized to all brain dopaminergic systems, since neither γ E nor Ac γ E affected the stereotyped sniffing response induced by apomorphine when injected directly into the nucleus caudatus.

The present studies show that γ E and Ac γ E differentially interact with distinct dopaminergic systems in the nucleus accumbens of the rat brain via an opioid and a non-opioid mechanism, suggesting that the peptide fragments originating from pro-opiomelanocortin may be specifically implicated in the control of dopaminergic activity in this brain area. Of particular interest is the dopaminergic system, which can be activated by low doses of apomorphine as well as bromocriptine (1 - 100 pg), and either directly or indirectly blocked by classical neuroleptic drugs (haloperidol, fluphenazine), atypical neuroleptic drugs (sulpiride, clozapine), and the γ -type endorphin peptides (γ E, Ac γ E, DT γ E, DE γ E) (7,14), which may be related to the established or purported anti-psychotic properties of these substances. Accordingly, certain dopaminergic systems in the nucleus accumbens have been implicated in the psychopathological syndrome schizophrenia (21). Since γ E and Ac γ E are present in the brain (15), one may speculate about a distinct physiological role of these peptides in the hormonal regulation of the activity of the different dopaminergic systems in the nucleus accumbens. As a consequence, disturbances in this hormonal control e.g. due to an altered ratio between the levels of γ E, Ac γ E and fragments

of these peptides, may eventually change the transmission of dopaminergic systems in that nucleus, which may be of relevance for the "so-called" dopamine hypothesis of schizophrenia (22,23). This suggestion agrees well with the original, more general, postulate that a relative deficiency of γ -type endorphins is a pathogenetic factor in the psychopathological syndrome schizophrenia (1,24)

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