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NON-OPIATE β-ENDORPHIN FRAGMENTS AND DOPAMINE—IV γ-TYPE ENDORPHINS MAY CONTROL DOPAMINERGIC SYSTEMS IN THE NUCLEUS ACCUMBENS

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Summary—Chronic treatment with des-enkephalin-γ-endorphin (DEγE, β-endorphin 6–17) twice daily for 10 days into the nucleus accumbens of rats resulted in hypoactivity, while similar treatment with γ-endorphin antiserum led to a marked hyperactivity. This enhanced activity persisted for at least 3 days following discontinuation of treatment. Rats chronically treated with γ-endorphin antiserum into the nucleus accumbens habituated at a slower rate when tested repeatedly for locomotor activity, as well as for nociception. Passive avoidance behaviour was attenuated in the treated rats, when they were trained during treatment, but not when the learning trial was given before treatment and testing was performed during treatment. Treatment with γ-endorphin antiserum did not affect the diurnal rhythm in locomotion, the responsiveness to nociceptive stimulation and the basal and novelty stress induced-plasma corticosteroid levels.

It is concluded that chronic treatment with γ-endorphin antiserum into the nucleus accumbens, which may lead to bio-inactivation of γ-type endorphins, causes hyperactivity and disturbances in habituation and cognitive functions. It is suggested that γ-type endorphins are physiologically involved in the control of distinct dopaminergic systems in the nucleus accumbens. The findings are discussed in relation to the role of mesolimbic dopaminergic systems in schizophrenic psychosis.

Previous experiments indicate that the neuroleptic-like and antipsychotic γ-type endorphins i.e. des-Tyr[1]-γ-endorphin (DTγE, β-endorphin (βE) 2–17) and des-enkephalin-γ-endorphin (DEγE, βE 6–17), given either subcutaneously or microinjected into the nucleus accumbens, counteract the hypoactivity induced by small doses of apomorphine in rats (Van Ree, Innemee, Louwerens, Kahn and De Wied, 1982a; Van Ree, Caffé and Wolterink, 1982b). From these data it was concluded that γ-type endorphins may act directly or indirectly as antagonists of distinct dopamine (DA) systems, which in the nucleus accumbens mediate apomorphine-induced hypoactivity. Further experiments revealed that subchronic treatment with DEγE injected into the nucleus accumbens for 4 days resulted in an increased sensitivity to apomorphine in that apomorphine-induced hypoactivity in the treated rats was enhanced instead of attenuated (Van Ree *et al.*, 1982b). Since DEγE and closely related peptides may be generated from β-endorphin to modulate DA activity (Verhoef, Loeber, Burbach, Gispen, Witter and De Wied, 1980; Burbach, Loeber, Verhoef, Wiegant, De Kloet and De Wied, 1980), it was postulated that a chronic deficiency of this peptide would lead to a state of subsensitivity of the DA systems mediating apomorphine-induced hypoactivity (Van Ree *et al.*, 1982b). Evidence has been presented that these DA systems may be located presynaptically on DA neurones involved in the control of locomotion and other behaviour. Subsensitivity of these DA systems would result in a sustained increase in DA activity and consequently in hyperactivity. To test this postulate a chronic deficiency of γ-type endorphins in the nucleus accumbens was experimentally induced by repeated local injection of γ-endorphin antiserum. The behavioural performance of the treated rats was analysed in a number of test paradigms. It was found that rats treated chronically with γ-endorphin antiserum into the nucleus accumbens show a long-lasting hyperactivity, habituate at a slower rate and are impaired in their cognitive capacities.

Key words: γ-type endorphins, des-enkephalin-γ-endorphin, DEγE, nucleus accumbens, dopamine, γ-endorphin antiserum, locomotion, habituation, cognitive function, passive avoidance behaviour, nociception, corticosteroids.

METHODS

Animals

Male rats of a Wistar strain, weighing 130–140 g at the time of operation, were used. They were equipped with a stainless steel cannula at each site of the brain and aimed at the nucleus accumbens. Details of the operation and housing conditions have been presented previously (Van Ree and Wolterink, 1981). Bilateral injections were given at 9.00 a.m. and 5.00 p.m. and testing was performed between 10.00 a.m. and 2.00 p.m. unless otherwise indicated. The volume of injection was always 1 μl. After experimentation the sites of injection were evaluated histologically as de-

scribed before (Van Ree and Wolterink, 1981). Data of rats with cannulae outside the nucleus accumbens were discarded from the analyses.

Test apparatus and procedures

(a) Small open field: a circular perspex test cage (diameter 19.5 cm, height 28.5 cm), the bottom of which was divided into 4 equal sections. Locomotor activity (number of sections explored at least with the fore legs) and rearing were observed for 3 min, unless otherwise indicated.

(b) Passive avoidance behaviour was studied in a simple step-through procedure as described elsewhere (Ader, Weijnen and Moleman, 1972). The apparatus consists of a dark box equipped with a grid floor and an illuminated, mesh-covered platform attached to the front center of the dark compartment. Rats were adapted to the dark box for 120 sec. Subsequently, the rats were placed on the runway and allowed to enter the dark compartment (first trial). Three such trials were given on the next day. Immediately after entering the dark compartment at the third trial, the rats received a single unavoidable scrambled footshock. Retention was tested twice, at 24 hr after the learning trial (first retention test) and later (second retention test). Latency to re-enter the dark compartment was recorded to a maximum of 300 sec.

(c) Responsiveness to electric footshock was studied according to the method of Gispen, Van Wimersma Greidanus and De Wied (1970). Two sets of 12 shocks intensities varying between 14 and 182 μA were used. Behavioural responses recorded at each shock level were flinch, jerk, jump, run and vocalizing (see also Mens and Van Ree, 1981).

(d) Hot plate device: response latency of rats was measured on a hot plate (56.0 $\pm$ 0.2°C) according to the method of Eddy and Leimbach (1953). The criterion of reaction of the rat was licking of one paw or intensive jerking with lifting off or jumping on hind legs. The test was terminated if the latency exceeded 60 sec.

(e) Corticosterone assay: corticosterone levels in plasma were measured after dilution in 0.2% ethyleneglycol and heat-inactivation (30 min, 80°C). A radioimmunoassay using an antiserum against corticosterone-21-hemisuccinate bovine serum (a gift from Dr Benraad, University of Nijmegen, The Netherlands) was performed to assess the corticosteroids.

Experiment 1

Groups of animals (n = 8–9) were injected bilaterally into the nucleus accumbens twice daily for 10 days with 10 ng DEγE dissolved in saline or placebo (1 μl saline). On the day following the last injection the rats were tested in the small open field and locomotor activity and rearing were observed for 3 min.

Experiment 2

Groups of animals (n = 6–10) were injected bilaterally into the nucleus accumbens twice daily for 10 or 11 days with 1 μl γ-endorphin antiserum (diluted 1:10 with saline) or placebo (1 μl normal rabbit serum, diluted 1:10 with saline). The animals treated for 10 days were tested on day 10 in the small open field while the animals treated for 11 days were tested on day 12 using the same procedure.

Experiment 3

Groups of animals (n = 14–18) were injected bilaterally into the nucleus accumbens twice daily for 12 days with γ-endorphin antiserum or placebo (see experiment 2). On day 11 of treatment the rats were tested twice in the small open field for 3 min with an intertrial interval of 20 min. On day 12 and 15 the rats were tested again in the small open field for 4 min. Locomotor activity and rearing were measured for the first and the second 2 min of the testperiod. On day 15 the activity of the rats during the first 3 min of the testperiod was also determined.

Experiment 4

Groups of animals (n = 13–17) were injected bilaterally into the nucleus accumbens twice daily for 12 days with γ-endorphin antiserum or placebo (see experiment 2). On day 11 of treatment approximately half of the rats of each treatment group was tested between 10.00 a.m. and 12.00 a.m., 4 times in the small open field for 3 min with an intertrial interval of 20 min. The other rats were tested once in the small open field between 8.00 and 10.00 p.m.

Experiment 5

Groups of rats (n = 6–9) at random selected from each treatment group of experiment 4 were tested for passive avoidance behaviour. Training started at 3 days before the treatment period. The learning trial (footshock 0.5 mA, 3″) was performed at 2 days and the first retention test at 1 day before treatment. The second retention test was on day 10 of treatment. Other groups of rats (n = 8–9) at random selected from the animals of experiment 3 were trained (footshock 0.25 mA, 2″) on day 11 of treatment and retention was measured on day 12 (last day of treatment) and on day 15 (3 days after treatment). Different shock intensities were used in the two experiments to account for the different time intervals between the first and second retention test.

Experiment 6

The rats of experiment 4 were randomly divided into two groups on day 12. One group of animals, treated with either placebo or γ-endorphin antiserum was tested for responsiveness to electric footshock. The other group was subjected to the hot plate procedure. In the latter test the animals received 4 trials with an intertrial interval of 20 min.

Experiment 7

On day 15 after completing behavioural testing, half of the rats of experiment 4 were decapitated and

blood was collected for measuring corticosteroid levels in plasma. The other rats were first placed for 5 min in a circular open field as described previously (Weijnen and Slangen, 1970) and decapitated immediately afterwards.

Peptide and rabbit serum

Des-enkephalin-γ-endorphin (DEγE, β-endorphin 6–17) was kindly donated by Dr H. M. Greven, Organon International B.V., Oss, The Netherlands. The characteristics of the antiserum have been presented previously (Loeber, Verhoef, Burbach and Witter, 1979). Briefly, des-Tyr^1-γ-endorphin and DEγE completely cross-react with γ-endorphin, while the cross-reactivity with β-endorphin, α-endorphin, des-Tyr^1-α-endorphin and met-enkephalin was low (4.8, 0.6, 0.02% respectively).

Analysis of the data

Groups mean ± SEM were calculated. Two tailed Student's (paired and non-paired) tests were used for statistical analysis of the data, except for the data obtained in the passive avoidance test for which the Mann–Whitney *U*-test was applied. When testing was performed more than twice the data were first analysed by two-way analysis of variance (ANOVA) testing.

RESULTS

The site of injection appeared to be in the middle and anterior part of the medial section of the nucleus accumbens on both sides (see Van Ree and Wolterink, 1981). In all experiments performed in the small open field, the duration of sniffing and the frequency of grooming, were measured in addition to locomotion and rearing. Sniffing and grooming occurred at a low level of frequency and was not different in placebo- and γ-endorphin antiserum-treated rats in any of the experiments.

Experiment 1

Chronic treatment with DEγE into the nucleus accumbens resulted in hypoactivity of the rats, when they were tested in a small open field. Both locomotion and rearing of these rats were significantly decreased as compared to those of placebo-treated controls (Fig. 1).

Experiment 2

Repeated injection of γ-endorphin antiserum into the nucleus accumbens led to hyperactivity of the animals. On the 10th day of treatment locomotor activity as well as rearing of these animals were markedly enhanced (Table 1). This increased activity was also present in animals tested approx. 19 hr after the last injection (Table 1).

Experiment 3

The hyperactivity of rats treated chronically with γ-endorphin antiserum into the nucleus accumbens as observed in experiment 2 was confirmed in this experiment (Fig. 2). In addition, it was found that placebo-treated rats habituated when they were tested again in the same test situation 20 min later. Thus, locomotion and rearing were significantly decreased during the second trial as compared to the first one (Fig. 2). In contrast, the locomotion of rats treated with γ-endorphin antiserum did not decline on repeated testing, while the rearing of these rats was slightly lower during the second trial. When the rats were tested again on the next day, the difference between γ-endorphin antiserum- and placebo-treated rats was even more pronounced, particularly with respect to locomotion (Table 2). The differences were present during the first as well as the second 2 min of testing. In both treatment groups the locomotion was significantly lower in the second part of the test period as compared to the first (Table 2). Three days after the last injection, the locomotor activity of the

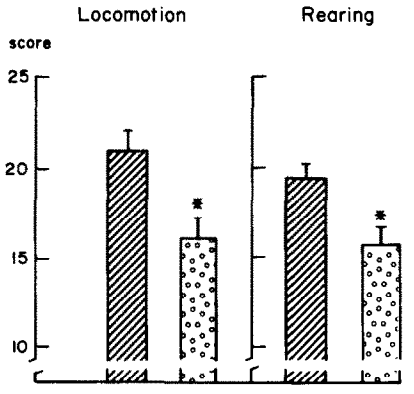

Fig. 1. Locomotion and rearing of rats treated twice daily with placebo (▨, 1 μl saline, $n = 8$) or des-enkephalin-γ-endorphin (DEγE, ▣, 10 ng, $n = 9$) injected into the nucleus accumbens for 10 days. Testing was performed on day 11 in a small open field for 3 min. Data are presented as mean score. Vertical bars indicate SEM. *Different from placebo treated rats ($P < 0.01$, Student's *t*-test).

Table 1. Locomotion and rearing of rats chronically treated with placebo or γ-endorphin antiserum injected into the nucleus accumbens

		Locomotion	Rearing
I.	Placebo	16.0 ± 1.0‡	14.9 ± 1.5 (10)
	Antiserum	26.7 ± 1.7**	21.7 ± 1.6* (7)
II.	Placebo	19.3 ± 0.7	19.8 ± 0.9 (6)
	Antiserum	24.7 ± 1.8*	22.7 ± 1.6 (7)

Rats were injected twice daily into the nucleus accumbens with 1 μl placebo (control rabbit serum diluted 1:10 with saline) or 1 μl γ-endorphin antiserum (diluted 1:10 with saline) for 10 days (I) or 11 days (II). Testing was performed on day 10 (I) or day 12 (II) in a small open field for 3 min.

* Different from placebo treated rats (*$P < 0.025$, **$P < 0.001$, Student's *t*-test).

‡ Mean ± SEM.

( ) Number of animals.

Table 2. Locomotion and rearing of rats chronically treated with placebo or γ-endorphin antiserum injected into the nucleus accumbens

		Locomotion			Rearing		
		Total score	1st part	2nd part	Total score	1st part	2nd part
I.	Placebo	16.2 ± 1.1‡	9.6 ± 0.7	6.6 ± 0.7††	11.0 ± 1.4	6.4 ± 0.8	4.7 ± 0.7†(17)
	Antiserum	33.3 ± 2.8**	18.2 ± 1.4**	15.1 ± 1.5**††	17.3 ± 2.0*	9.6 ± 1.2*	7.7 ± 1.2*(14)
II.	Placebo	17.1 ± 0.9	10.5 ± 0.6	6.5 ± 0.5††	9.1 ± 1.2	5.9 ± 0.8	3.2 ± 0.6††
	Antiserum	26.8 ± 2.9*	16.2 ± 1.4**	10.6 ± 1.8*†	12.6 ± 1.9	7.8 ± 1.3	4.8 ± 1.0†

Rats were injected twice daily into the nucleus accumbens with 1 μl placebo (control rabbit serum, diluted 1:10 with saline) or 1 μl γ-endorphin antiserum (diluted 1:10 with saline) for 12 days. Testing was performed on day 12 (I) and on day 15 (3 days after treatment, II) in a small open field for 4 min. Locomotion and rearing were scored during the first and second 2 min of the test period.

* Different from placebo treated rats (*$P < 0.05$, **$P < 0.001$, Student's t-test).
† Different from score in the first part of the testperiod (†$P < 0.05$, ††$P < 0.001$, Student's t-test).
‡ Mean ± SEM.
( ) Number of animals.

rats treated with γ-endorphin antiserum was still enhanced as compared to that of placebo-treated controls. Similar differences with respect to the first and second part of the testperiod as noticed on the last day of treatment were present 3 days after treatment (Table 2). Comparing the data obtained on day 11 of treatment and on day 15 (3 days after treatment), it was found that the locomotion of placebo-treated rats was more decreased than that of rats injected with γ-endorphin antiserum (placebo $t = 8{,}814$, $P < 0.001$; γ-endorphin antiserum: $t = 2{,}57$, $P < 0.025$, testing was performed on the data of the first 3 min of testing using the Student's paired t-test).

Experiment 4

In order to study the influence of chronic treatment with γ-endorphin antiserum injected into the accumbens on habituation in more detail, rats were tested 4 times in the small open field with an intertrial interval of 20 min. Placebo-treated rats already habituated to the test situation at the second test trial, in that both locomotion and rearing were lower as compared to those of the first trial, as observed in experiment 3. In contrast, the scores obtained in the γ-endorphin antiserum treated rats at the second trial hardly differed from those of the first trial (Fig. 3). On subsequent testing in both treatment groups, locomotion and rearing decreased, although the rate of habituation, as indicated by the F value of ANOVA testing, was lower in the γ-endorphin antiserum-treated rats than in control animals.

When testing was performed in the dark phase, an increased locomotion and rearing was observed in the placebo-treated rats as compared to rats tested in the light phase (Table 3). A similar and even more pronounced difference was noted in rats treated with γ-endorphin antiserum.

Experiment 5

Rats subjected to passive avoidance training before the treatment period showed a rather long latency to re-enter the dark compartment during the first retention trial, which was also performed before treatment with γ-endorphin antiserum (Fig. 4). This latency was less at the second retention test which was performed after 10 days of treatment. No differences were observed between the performance of rats treated with placebo or γ-endorphin antiserum. However, when the learning trial was given on day 11 of treatment, passive avoidance behaviour was markedly attenuated in rats treated with γ-endorphin antiserum. This was apparent from the first as well as from the second retention test (Fig. 4).

Experiment 6

Nociception of rats was not affected by chronic treatment into the accumbens with γ-endorphin antiserum. Neither the responsiveness to electric footshock nor the response-latency in the hot plate at the first test trial was changed in rats treated with

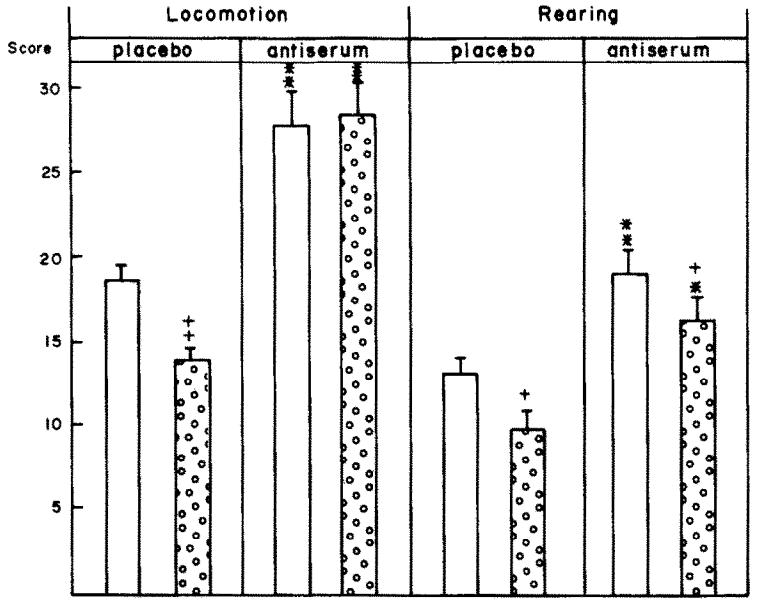

Fig. 2. Locomotion and rearing of rats treated twice daily with 1 μl placebo (control rabbit serum, diluted 1:10 with saline, $n = 18$) or 1 μl γ-endorphin antiserum, diluted 1:10 with saline, $n = 14$) injected into the nucleus accumbens for 12 days. Testing was performed on day 11 twice in a small open field for 3 min with a time interval of 20 min (□ first test trial, ⊡ second test trial). Data are presented as mean score. Vertical bars indicate SEM. *Different from placebo treated rats ($*P < 0.005$, $**P < 0.001$, Student's t-test). †Different from score at first test trial ($\dagger P < 0.01$, $\dagger\dagger P < 0.001$, Student's paired t-test).

γ-endorphin antiserum as compared to placebo-treated controls (Table 4). On repeated testing on the hot plate, it was found that the decrease of response latency over time was more pronounced in the placebo-treated rats than in those treated with γ-endorphin antiserum. In fact, a significant difference between the first and second test trial was present in the placebo-treated rats, while such a difference was not observed

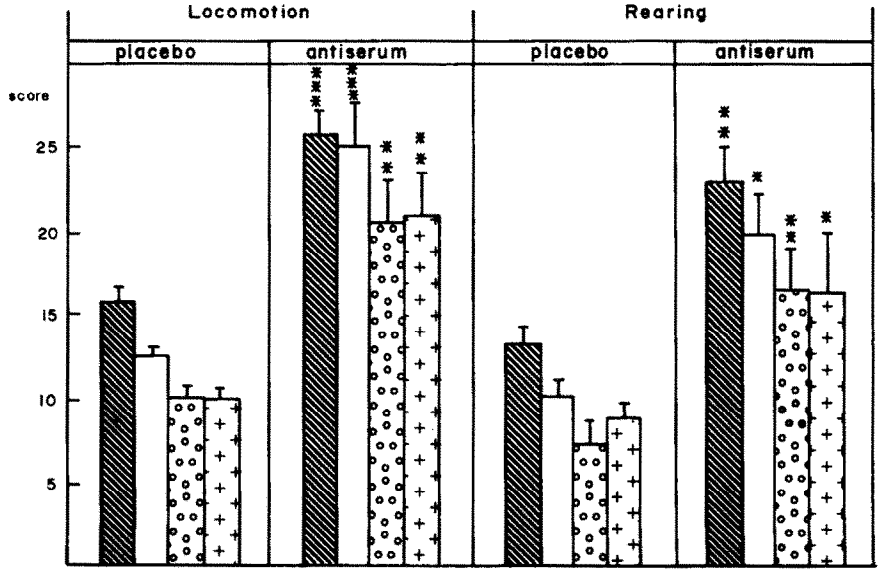

Fig. 3. Locomotion and rearing of rats treated twice daily with 1 μl placebo (control rabbit serum, diluted 1:10 with saline, $n = 8$) or 1 μl γ-endorphin antiserum, diluted 1:10 with saline, $n = 7$) injected into the nucleus accumbens for 12 days. Testing was performed on day 11 four times in a small open field for 3 min with an intertrial interval of 20 min (▨ first test trial, □ second test trial, ⊡ third test trial, ⊞ fourth test trial). Data are presented as mean score. Vertical bars indicate SEM. *Different from placebo treated rats ($*P < 0.05$, $**P < 0.005$, $***P < 0.001$). Two way ANOVA testing: locomotion: placebo $F = 19.07$, antiserum $F = 9.83$; rearing: placebo $F = 9.83$, antiserum $F = 6.77$.

Table 3. Locomotion and rearing of rats chronically treated with placebo or γ-endorphin antiserum injected into the nucleus accumbens and tested during the light or the dark phase

	Locomotion		Rearing	
	Light	Dark	Light	Dark
Placebo	15.8 ± 0.8‡ (8)	21.0 ± 0.8†† (9)	13.2 ± 1.1	18.8 ± 1.1††
Antiserum	25.7 ± 1.4** (7)	39.3 ± 3.4**†† (6)	22.9 ± 2.2*	37.2 ± 5.0**†

Rats were injected twice daily into the nucleus accumbens with 1 μl placebo (control rabbit serum, diluted 1 : 10 with saline) or 1 μl γ-endorphin antiserum (diluted 1 : 10 with saline) for 12 days. Testing was performed on day 11 in the light phase (between 10.00 and 12.00 a.m.) or in the dark phase (between 8.00 and 10.00 p.m.) in a small open field for 3 min.

* Different from placebo treated rats (*$P < 0.005$, **$P < 0.001$, Student's t-test).

† Different from the rats tested during the light phase (†$P < 0.02$, ††$P < 0.005$, Student's t-test).

‡ Mean ± SEM.

( ) Number of animals.

in the rats treated with γ-endorphin antiserum before the fourth test-trial (Table 4).

Experiment 7

Three days after treatment no differences in basal plasma corticosterone levels were found between rats treated with γ-endorphin antiserum or vehicle for 12 days (Table 5). Placing the animals in a novel situation increased plasma corticosterone levels. This increase was similar in placebo- and γ-endorphin antiserum-treated animals (Table 5).

DISCUSSION

The present data show that chronic treatment with the neuroleptic-like and antipsychotic peptide desenkephalin-γ-endorphin (DEγE, βE 6–17) injected into the nucleus accumbens led to hypoactivity of rats and that similar treatment with γ-endorphin antiserum resulted in hyperactivity. Since dopamine (DA) systems in the nucleus accumbens have been implicated in the control of locomotion (Costall, Naylor, Cannon and Lee, 1977; Pijnenburg, Honig and Van Rossum, 1975; Pijnenburg, Honig, Van der Heijden and Van Rossum, 1976) and γ-type endorphin are present in this nucleus (Dorsa, Majumdar and Chapman, 1982), DEγE and related peptides may be physiologically involved in the control of certain DA systems in the nucleus accumbens. It has been argued that (sub)chronic treatment with DEγE may result in the development of supersensitivity of DA receptor systems, mediating the hypoactivity induced by sub-

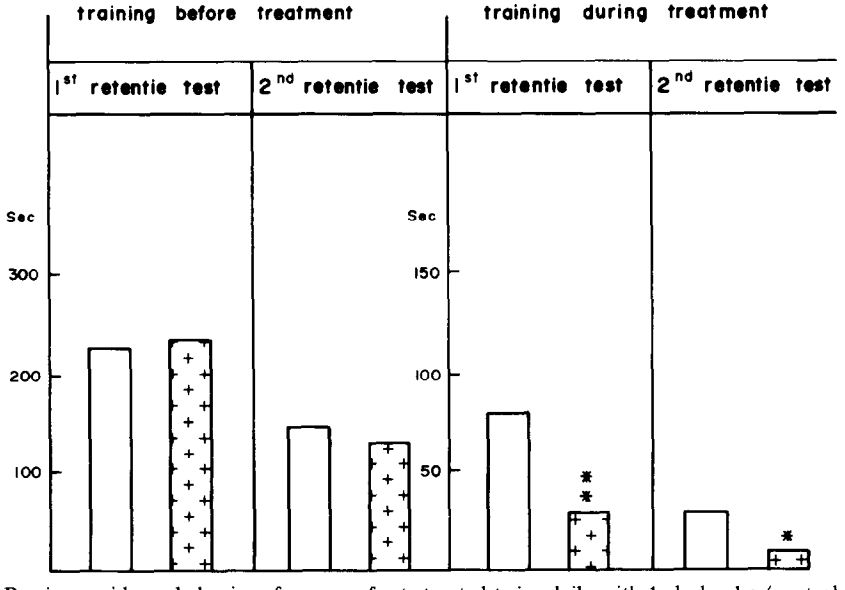

Fig. 4. Passive avoidance behavior of groups of rats treated twice daily with 1 μl placebo (control rabbit serum, diluted 1:10 with saline, □) or 1 μl γ-endorphin antiserum (diluted 1:10 with saline ⊞), injected into the nucleus accumbens for 12 days. In the first experiment the learning trial (footshock 0.5 mA, 3″) was given 2 days before the start of the treatment period and retention was tested at 1 day before treatment (first) and on day 10 of treatment (second). In the second experiment the learning trial (footshock 0.25 mA, 2″) was given on day 11 of treatment and retention was tested on day 12 of treatment (first) and on day 15 (3 days after treatment, second). Results are given as median values (sec) of groups of 6–9 rats. *Different from placebo treated rats (*$P < 0.05$, **$P < 0.002$, Mann–Whitney U-test).

Table 4. Responsiveness to nociceptive stimulation of rats chronically treated with placebo or γ-endorphin antiserum injected into the nucleus accumbens

(A) Electric footshock procedure						
Response	No response	Flinch	Jerk	Run	Jump	Vocalization
Placebo (7)	1.7 ± 0.5	7.3 ± 0.6	13.6 ± 0.6	1.4 ± 0.6	0	12.0 ± 1.3
Antiserum (6)	2.0 ± 0.5	5.7 ± 0.7	13.3 ± 1.5	2.0 ± 1.1	1.0 ± 0.8	9.8 ± 1.3
(B) Hot plate procedure						
Test trial	1st	2nd	3rd	4th		
Placebo (9)	6.5 ± 0.5	4.9 ± 0.4*	5.7 ± 0.6	4.4 ± 0.3*		
Antiserum (7)	6.4 ± 0.4	6.2 ± 0.6	6.4 ± 0.4	5.2 ± 0.3*		

Rats were injected twice daily into the nucleus accumbens with 1 μl placebo (control rabbit serum, diluted 1:10 with saline) or 1 μl γ-endorphin antiserum (diluted 1:10 with saline) for 12 days. On day 12 the rats were subjected to either the electric footshock procedure (A) or the hot plate procedure (B). The results of the electric footshock procedure are given as mean (± SEM) of occurrence of a certain response on presentation of each of the 24 shock intensities (for definition of the behavioral responses see Mens and Van Ree, 1981). The data of the hot plate procedure are presented as the mean latency (sec ± SEM) to the first reaction of the rat to the nociceptive stimulation. The rats were four times tested on the hot plate with an intertrial interval of 20 min. Two way ANOVA testing on these data indicate an effect of repeated testing in the placebo treated rats ($F = 6{,}233$ (3,24) $P < 0.01$), while this effect was not present in the antiserum treated rats ($F = 3{,}047$ (3,18) $P > 0.05$).

* Different from the results of the first test trial ($P < 0.01$, Student's paired t-test).

( ) Number of rats.

cutaneous as well as injections into the accumbens of small doses of apomorphine (Van Ree *et al.* 1982a, b). These receptor systems may be located presynaptically in the DA neurones involved in the control of locomotion. Consequently, supersensitivity of these DA systems of the accumbens may result in a diminished DA release causing hypoactivity. Conversely, subsensitivity of these receptor systems may lead to an enhanced DA release and to hyperactivity. Thus, the effects of chronic treatment with γ-endorphin antiserum injected into the accumbens may be explained by subsensitivity of DA receptor systems located presynaptically on DA neurones. However, the consequence of this subsensitivity, i.e. an increased DA transmission, has to be demonstrated before definite conclusions can be drawn.

To induce a chronic deficiency of γ-type endorphins in the nucleus accumbens, γ-endorphin antiserum was used. This antiserum is specific for γ-type endorphins, at least as far as its application in radioimmunoassay procedures is concerned, in that radioactive γ-endorphin is displaced from the antiserum by γ-type endorphins and not by β-endorphin and α-type endorphins (Loeber *et al.*, 1979). However, its use *in vivo* is much more complicated, since the antiserum binds α- and β-endorphin in addition to γ-endorphin (Loeber, personal communication). In fact, the capacity of the antiserum to bind α, β and γ-endorphin was 200, 966 and 1333 fmol/μl respectively. Moreover, the antiserum may induce effects which are not related to bioinactivation of γ-type endorphins. However, α-type endorphins and β-endorphin do not interfere with apomorphine-induced hypoactivity (Van Ree, 1982; Van Ree *et al.*, 1982b; Van Ree, unpublished data). The striking opposing effects of chronic treatment with DEγE injected into the accumbens and that with γ-endorphin antiserum, suggest that the effects of the antiserum are due to a deficiency in γ-type endorphins.

Table 5. Plasma corticosterone (CS) levels in rats chronically treated with placebo or γ-endorphin antiserum injected into the nucleus accumbens

	CS levels (μg/100 ml)	
	Basal	After novel situation
Placebo	10.4 ± 2.8‡ (8)	21.0 ± 2.4* (8)
Antiserum	11.9 ± 2.2 (7)	21.1 ± 3.7* (6)

Rats were injected twice daily into the nucleus accumbens with 1 μl placebo (control rabbit serum, diluted 1:10 with saline) or 1 μl γ-endorphin antiserum (diluted 1:10 with saline) for 12 days. Three days after treatment groups of rats were decapitated and basal plasma CS levels were assessed. In other groups CS levels were determined after placing the rats for 5 min in a novel open field.

* Different from basal levels ($P < 0.05$, Student's t-test).

‡ Mean ± SEM.

( ) Number of animals.

Mesolimbic DA systems especially in the nucleus accumbens may be concerned in exploratory behaviour in response to novel stimuli (Fink and Smith, 1980), displacement behaviour, which may develop in part by effects of non-specific motivational excitement (Robbins and Koob, 1980), cognitive and attentional processes (Simon, Scatton and Le Moal, 1980) and are selectively activated by electric footshock stress (Thierry, Tassin, Blanc and Glowinski, 1976). It has been suggested that the nucleus accumbens may be part of a functional link between limbic and motor systems (Nauta, Smith, Faull and Domesick, 1978; Jones, Magenson and Wu, 1981). In view of the suggestion that chronic treatment with γ-endorphin antiserum may eventually lead to an increased DA transmission in the nucleus accumbens, the behavioural performance of rats treated in this way was

analysed in detail, using test procedures more or less selective for the functions in which nucleus accumbens DA systems seem to be involved. These studies revealed that rats treated chronically with γ-endorphin antiserum injected into the nucleus accumbens showed a profound and sustained hyperactivity which lasted at least 72 hr after discontinuation of treatment and habituated and/or adapted to a test situation (i.e. open field, hotplate) at a slower rate than control rats. These rats did not show disturbances in the diurnal rhythm in locomotor activity. Interestingly, these animals displayed an attenuated passive avoidance response when learning and testing took place during chronic treatment, but showed a normal response when the learning trial was given before treatment and testing was performed during treatment. Finally, the rats responded normally to nociceptive stimulation and had unchanged basal and novelty stress-induced plasma corticosterone levels. The decreased habituation could have been explained by disturbances in perception, as awareness of sensory stimuli. However, this is less likely, since the rats showed a normal response to nociceptive stimulation and decreased their locomotion in a single test trial (Table 2). It is more likely that the rats had difficulties in remembering prior experiences. Thus, the interpretation following the perception of sensory stimuli may be particularly disturbed in rats treated with γ-endorphin antiserum. Also the data of passive avoidance behaviour point to disturbances in cognitive functions of these rats. Since retrieval of information, given prior to treatment, was not affected and nociceptive stimuli seemed to be perceived in a normal way, the rats treated with γ-endorphin antiserum may be impaired in their cognitive capacities. Although the mesolimbic DA system is selectively activated by stress (Thierry *et al.*, 1976), it is rather unlikely that stress factors are primarily involved in the disturbed behaviour of the rats, since both basal and novelty stress-induced increase in plasma corticosterone levels were unchanged after chronic treatment with γ-endorphin antiserum.

In view of the postulate that a deficiency of γ-type endorphins may be an etiological factor in schizophrenic psychosis (De Wied, 1978), and that the mesolimbic DA systems may be involved in this mental disorder (Crow, 1979), a deficiency of γ-type endorphins in mesolimbic DA systems was induced to characterize the behavioural performances of these animals. The present data suggest that rats treated chronically with γ-endorphin antiserum into the nucleus accumbens have difficulties in habituation and/or adaptation and are impaired in cognitive capacities, disturbances which are not uncommon in schizophrenic patients. However, more studies are needed both in animals and in patients, to relate a deficiency in γ-type endorphins to psychotic symptoms of schizophrenic patients. Nevertheless, the present data favour the suggestion that a deficient neuroendocrine control over mesolimbic DA systems may be involved in the ethiology of schizophrenic psychosis (Stevens, 1979).

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REFERENCES

Ader, R., Weijnen, J. A. W. M. and Moleman, P. (1972). Retention of a passive avoidance response as a function of the intensity and duration of electric shock. *Psycho. Sci.* **26:** 125–128.

Burbach, J. P. H., Loeber, J. G., Verhoef, J., Wiegant, V. M., De Kloet, E. R. and De Wied, D. (1980). Selective conversion of β-endorphin into peptides related to γ- and α-endorphin. *Nature* **283:** 96–97.

Costall, B., Naylor, R. J., Cannon, J. G. and Lee, T. (1977). Differentiation of the dopamine mechanisms mediating stereotyped behaviour and hyperactivity in the nucleus accumbens and caudate–putamen. *J. Pharm. Pharmac.* **29:** 337–342.

Crow, T. J. (1979). Dopaminergic mechanisms in schizophrenia: site and mechanisms of antipsychotic effect and postmortem studies. In: *Neuroleptics and Schizophrenia* (Simister, J. M., Ed.), pp. 29–40. Lundbeck, Luton.

De Wied, D. (1978). Psychopathology as a neuropeptide dysfunction. In: *Characteristics and Function of Opioids* (Van Ree, J. M. and Terenius, L., Eds), pp. 113–122. Elsevier, Amsterdam.

Dorsa, D. M., Majumdar, L. A. and Chapman, M. B. (1982). Regional distribution of gamma and beta-endorphin-like peptides in the pituitary and brain of the rat. *Peptides* (in press).

Eddy, N. B. and Leimbach, D. (1953). Synthetic analgesics II, dithienylbutenyl- and dithienylbutylamines. *J. Pharmac. exp. Ther.* **107:** 385–393.

Fink, J. S. and Smith, G. P. (1980). Mesolimbicocortical dopamine terminal fields are necessary for normal locomotor and investigatory exploration in rats. *Brain Res.* **199:** 359–384.

Gispen, W. H., Van Wimersma Greidanus, Tj. B. and De Wied, D. (1970). Effects of hypophysectomy and ACTH 1–10 on responsiveness to electric shock in rats. *Physiol. Behav.* **5:** 143–146.

Jones, D. L., Mogensen, G. J. and Wu, M. (1981). Injections of dopaminergic, cholinergic, serotoninergic and gabaergic drugs into the nucleus accumbens: effects on locomotor activity in the rat. *Neuropharmacology* **20:** 29–37.

Loeber, J. G., Verhoef, J., Burbach, J. P. H. and Witter, A. (1979). Combination of high pressure liquid chromatography and radioimmunoassay is a powerful tool for the specific and quantitative determination of endorphins and related peptides. *Biochem. biophys. Res. Commun.* **86:** 1288–1295.

Mens, W. B. J. and Van Ree, J. M. (1981). Influence of β-lipotropin fragments on responsiveness of rats to electric footshock. *Pharmac. Biochem. Behav.* **15:** 27–32.

Nauta, W. J. H., Smith, G. P., Faull, R. L. M. and Domesick, V. B. (1978). Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. *Neuroscience* **3:** 385–401.

Pijnenburg, A. J. J., Honig, W. M. M. and Van Rossum, J. M. (1975). Inhibition of *d*-amphetamine-induced locomotor activity by injection of haloperidol into the nucleus accumbens of the rat. *Psychopharmacologia* **41:** 87–95.

Pijnenburg, A. J. J., Honig, W. M. M., Van der Heyden, J. A. M. and Van Rossum, J. M. (1976). Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. *Eur. J. Pharmac.* **35:** 45–58.

Robbins, T. W. and Koob, G. F. (1980). Selective disruption of displacement behaviour by lesions of the mesolimbic dopamine system. *Nature* **285:** 409–412.

Simon, H., Scatton, B. and Le Moal, M. (1980). Dopaminergic A10 neurones are involved in cognitive functions. *Nature* **286:** 150–151.

Stevens, R. C. (1979). Schizophrenia and dopamine regulation in the mesolimbic system. *Trends Neurosci.* **2:** 102–105.

Thierry, A. M., Tassin, J. P., Blanc, G. and Glowinski, J. (1976). Selective activation of the mesocortical DA system by stress. *Nature* **263:** 242–244.

Van Ree, J. M. (1982). Non-opiate β-endorphin fragments and dopamine—II: β-Endorphin 2–9 enhances apomorphine-induced stereotype following subcutaneous and intra-striatal injection. *Neuropharmacology* **21:** 1103–1109.

Van Ree, J. M. and Wolterink, G. (1981). Injection of low doses of apomorphine into the nucleus accumbens of rats reduces locomotor activity. *Eur. J. Pharmac.* **72:** 107–111.

Van Ree, J. M., Innemee, H., Louwerens, J. W. Kahn, R. S. and De Wied, D. (1982a). Non-opiate β-endorphin fragments and dopamine—I: The neuroleptic-like γ-endorphin fragments interfere with behavioral effects elicited by small doses of apomorphine. *Neuropharmacology* **21:** 1095–1101.

Van Ree, J. M., Caffé, A. R. and Wolterink, G. (1982b). Non-opiate β-endorphin fragments and dopamine—III: γ-Type endorphins and various neuroleptics counteract the hypoactivity elicited by intra-accumbens injection of apomorphine. *Neuropharmacology* **21:** 1111–1117.

Verhoef, J., Loeber, J. G., Burbach, J. P. H., Gispen, W. H., Witter, A. and De Wied, D. (1980). α-Endorphin, γ-endorphin and their des-tyrosine fragments in rat pituitary and brain tissue. *Life Sci.* **26:** 851–859.

Weijnen, J. A. W. M. and Slangen, J. L. (1970). Effects of ACTH-analogues on extinction of conditioned behavior. In: *Pituitary, Adrenal and the Brain* (De Wied, D. and Weijnen, J. A. W. M., Eds), *Progr. Brain Res.* Vol. 32, pp. 221–235. Elsevier, Amsterdam.