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Behavioral studies on the putative γ-type endorphin receptor using different antibodies

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Abstract

To investigate the significance of endogenous, neuroleptic-like γ-type endorphins and their putative receptors, polyclonal and monoclonal antibodies against γ-type endorphins, which may bio-inactivate the ligands for the receptors, and monoclonal anti-idiotype antibodies, which presumably bind to the receptors, were injected into the nucleus accumbens of the rat brain. The desenkephalin-γ-endorphin-induced antagonism of the hypomotility response elicited by challenge with apomorphine injected into the nucleus accumbens was used as test system. Both the anti-desenkephalin-γ-endorphin antibodies and anti-idiotype antibodies blocked the action of exogenous desenkephalin-γ-endorphin. Thus, the anti-idiotype antibodies may serve as receptor antagonists. Chronic treatment (injection into the nucleus accumbens) with the anti-idiotype antibodies induced sustained hypermotility, decreased habituation and impaired passive avoidance behavior. In such treated animals local treatment with apomorphine did not elicit hypomotility. It is suggested that γ-type endorphins influence the setpoint for feedback regulation in dopaminergic neurons equipped with γ-type endorphin receptor systems.

Keywords: Desenkephalin-γ-endorphin; Endorphin receptor, γ-type; Apomorphine; Nucleus accumbens; Neuroleptic-like action; Antibody; Anti-idiotype antibody; Behavioral effect

1. Introduction

The endogenous opioid β-endorphin can be proteolytically processed to yield the opioids γ-endorphin and α-endorphin, as well as non-opioid fragments of these peptides (Burbach et al., 1980, 1981; Burbach and Wiegant, 1990). From behavioral studies using a variety of test procedures, it has been concluded that γ-type endorphins induce effects that resemble those observed after treatment with neuroleptics, while α-type endorphins exert effects that can also be induced by psychostimulants like amphetamine (De Wied et al., 1978, 1980; Van Ree et al., 1980, 1982a; Van Ree and De Wied, 1982). These observations led to the postulate that γ-type endorphins are endogenous peptides with neuroleptic-like activities (De Wied, 1978). γ-Type endorphins are indeed present in the brain (Dorsa et al., 1982; Dorsa and Majumdar, 1983; Wiegant et al., 1983, 1985, 1988). Clinical trials have shown that γ-type endorphins (Des-Tyr^1-γ-endorphin and desenkephalin-γ-endorphin) induce antipsychotic effects in a category of schizophrenic patients (Verhoeven et al., 1979; Van Ree et al., 1987). In view of the dopamine hypothesis of schizophrenia (Matthysse, 1974; Meltzer and Stahl, 1976; Van Kammen, 1979; Crow, 1979), a series of studies was performed aimed at the interaction between γ-type endorphins and brain dopaminergic systems, in order to unravel the mode of action of these peptides (Van Ree and De Wied, 1982). One of the test models used was the injection of the dopamine agonist apomorphine into the different terminal areas of brain dopaminergic systems. Locally injected desenkephalin-γ-endorphin, the shortest γ-type endorphin with neuroleptic-like activity after peripheral administration, dose dependently antagonized the hypomotility

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induced by low doses of apomorphine injected into the nucleus accumbens and the stereotyped sniffing induced by low and high doses of apomorphine injected in the pyriform cortex (Van Ree et al., 1989). However, no antagonism was found when the peptide and high doses of apomorphine were injected into the nucleus caudatus or the nucleus accumbens. Since dopaminergic systems in the nucleus accumbens have been implicated in the antipsychotic action of neuroleptics (Stevens, 1979), this area was selected for further studies (Van Ree et al., 1982b,c; Radhakishun and Van Ree, 1987; Radhakishun et al., 1988). The antipsychotic effects of neuroleptics and γ-type endorphins in schizophrenic patients are observed following repeated administration (Verhoeven et al., 1979, 1982), and the schizophrenic disease is a chronic disorder. This prompted studies with chronic treatment with desenkephalin-γ-endorphin and antibodies against γ-type endorphins. Chronic treatment with desenkephalin-γ-endorphin injected into the nucleus accumbens induced hypomotility and treatment with γ-endorphin antibodies elicited hypermotility (Van Ree et al., 1982c). The release of dopamine from nucleus accumbens tissue in vitro and in vivo determined using a push pull cannula in the nucleus accumbens, appeared to be decreased following chronic peripheral administration of desenkephalin-γ-endorphin (Radhakishun et al., 1994).

Recently, it was shown that the nucleus accumbens possessed high affinity binding sites for [^{35}S]Met-desenkephalin-γ-endorphin (Ronken et al., 1989, 1993). This finding, together with the behavioral effects of desenkephalin-γ-endorphin, may be evidence for the existence of γ-type endorphin receptors. To further investigate the function of this putative receptor system in the nucleus accumbens, a series of studies was performed using polyclonal and monoclonal antibodies specifically directed against the C-terminus of γ-type endorphins and a monoclonal anti-idiotype antibody (raised against the idiotype of a monoclonal desenkephalin-γ-endorphin antibody) with high affinity for desenkephalin-γ-endorphin-binding sites in the brain (Ronken et al., 1993). It was reasoned that desenkephalin-γ-endorphin antibodies can neutralize the receptor ligand and that the anti-idiotype antibody would bind to the receptor without leading to receptor activation, thereby functioning as a receptor antagonist.

2. Material and methods

2.1. Animal and housing conditions

Male Wistar rats from our own stock were used. They weighed between 150 and 180 g at the time of testing. The animals were housed in a dimly lit animal room (light on 6.00 a.m., off 8.00 p.m.) in groups of 5–6 animals per cage (40 × 26 × 20 cm, l × w × h). They had free access to food and tap water. One day prior to testing the animals were handled.

2.2. Surgical and injection procedure

The rats were anesthetized with Hypnorm (0.1 ml per 100 g body weight) and secured in a stereotaxic instrument. Stainless steel cannulae (0.6 mm outer diameter, 0.3 mm inner diameter) were implanted bilaterally in the nucleus accumbens. The coordinates were 2.6 mm anterior to bregma, 2.7 mm lateral to the midline, 6.2 mm below the surface of the skull at the point of penetration, inserted at an angle of 12°, incision bar at horizontal zero level. The cannulae were secured to the skull with stainless steel screws and dental cement to cover the area of surgery. Stainless steel occluders were placed in the cannulae to ensure their patency. The rats were allowed to recover from the operation for at least 7 days. Injections through the cannulae were given by using a SGE glass microsyringe (0.25 mm outer diameter). The rats were hand-restrained, not anesthetized, during injections. The occluder was removed and the microsyringe with the correct length for the guide cannula was inserted. All injections were performed bilaterally. A volume of 1.0 μl was injected in 30 s and after 1 min the occluder was reinserted. The occluder fully filled the guide cannula. Desenkephalin-γ-endorphin treatment was always followed by an injection with apomorphine, since this was pertinent to the tested hypothesis and acute treatment with desenkephalin-γ-endorphin did not affect motility in the test procedure used (Van Ree et al., 1982b, 1989). The time of injection before testing and the amount of apomorphine and desenkephalin-γ-endorphin injected were adopted from previous experiments (Van Ree and Wolterink, 1981; Van Ree et al., 1982b,c, 1989).

2.3. Behavioral testing

The behavioral tests were performed by different observers (G.W., Y.I. and L.V.). No difference in results was noticed among the observers.

Motor activity

On the day of behavioral testing the animals were brought to the sound-attenuated observation room at least 1 h prior to testing. The behaviors were assessed in a small open field, consisting of a transparent Plexiglas tube (diameter 19.5 cm, height 30 cm) placed on a plastic board which was divided into four equal sections, each with a surface of about 75 cm^2. The rats were placed in the middle of the small open field just

before testing started. During a 3 min observation period the number of sections explored at least with the forelegs (motor activity) was measured. The rats were tested only once, unless otherwise indicated. Testing was performed between 10.00 a.m. and 2.00 p.m.

Passive avoidance behavior

Passive avoidance behavior was studied in a simple step-through procedure as described elsewhere (Ader et al., 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. The rats were adapted to the dark box for 120 s. Subsequently, they 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 in the third trial, the rats received a single unavoidable scrambled footshock (0.25 mA, 2 s). Retention was tested 24 h after the learning trial. Latency to re-enter the dark compartment was recorded to a maximum of 300 s.

2.4. Experimental procedures

Experiment 1

The first experiment was designed to test whether the polyclonal γ-endorphin antiserum could block the attenuating effect of desenkephalin-γ-endorphin on apomorphine-induced hypomotility. Besides control rabbit serum, oxytocin antiserum was tested in order to investigate the specificity of the γ-endorphin antiserum. Fifteen groups of animals (6–15 animals per group) were tested. The rats received 3 injections before testing in the small open field. The first injection was control rabbit serum diluted 1:10 or 1:100 with saline (control injection); rabbit polyclonal γ-endorphin antiserum (L10) diluted 1:10 or 1:100 with saline; or oxytocin antiserum diluted 1:10 with saline. After 10 min, saline or desenkephalin-γ-endorphin (100 pg) was injected. This was followed by an injection of saline or apomorphine (10 ng) after 40 min. Desenkephalin-γ-endorphin-treated rats all received apomorphine. The rats were behaviorally tested 20 min after the last injection.

Experiment 2

In this experiment the effect of monoclonal desenkephalin-γ-endorphin antibody on the attenuating effect of desenkephalin-γ-endorphin on apomorphine-induced hypomotility was investigated. Six groups of animals (6–10 animals per group) were tested. The rats received 3 injections before testing in the small open field. The first injection was saline or rat monoclonal desenkephalin-γ-endorphin antibody (CR1B3). After 10 min saline or desenkephalin-γ-endorphin (100 pg) was injected, followed by injection of saline or apomorphine (10 ng) after 40 min. Desenkephalin-γ-endorphin-treated rats all received apomorphine. The rats were behaviorally tested 20 min after the last injection.

Experiment 3

Next, the effect of two preparations of monoclonal anti-idiotype antibody on the attenuating effect of desenkephalin-γ-endorphin on apomorphine-induced hypomotility was investigated. Nine groups of animals (5–12 animals per group) were tested. The rats received 3 injections before testing in the small open field. The first injection was saline or rat monoclonal anti-idiotype antibody (CR22 or CR14), the second after 10 min consisted of saline or desenkephalin-γ-endorphin (100 pg) and the third saline or apomorphine (10 ng) given 40 min after the second injection. Desenkephalin-γ-endorphin-treated rats all received apomorphine. The rats were behaviorally tested 20 min after the last injection.

Experiment 4

The anti-idiotype antibody CR14 was used for further experimentation. To investigate the influence of protein material present in the preparation, the effect of CR14 was compared to that of a dialyzed preparation. Four groups of animals ($n = 6–8$) were bilaterally injected with saline, monoclonal anti-idiotype antibody (CR14), dialyzed monoclonal anti-idiotype antibody (d-CR14) and the dialyzed control solution (d-control). After 10 and 50 min the animals were injected with desenkephalin-γ-endorphin (100 pg) and apomorphine (10 ng) respectively. The rats were behaviorally tested 20 min after the last injection.

Experiment 5

In this experiment, graded dilutions of the dialyzed monoclonal anti-idiotype antibody were investigated. Eight groups of animals (5–13 animals per group) were tested. The rats received 3 injections before testing in the small open field. The first injection was saline or dialyzed monoclonal anti-idiotype antibody (d-CR14) diluted 1:10, 1:100, 1:1000, 1:10000 or 1:100000 with saline. After 10 min saline or desenkephalin-γ-endorphin (100 pg) was injected, followed by saline or apomorphine (10 ng) after 40 min. All rats treated with antibody received desenkephalin-γ-endorphin and apomorphine. The rats were behaviorally tested 20 min after the last injection.

Experiment 6

The data of experiment 5 indicated that a dilution of 10^4 of the d-CR14 preparation was effective in antagonizing the effect of desenkephalin-γ-endorphin. This dilution was used in experiment 6, which investigated whether an increasing dose of desenkephalin-γ-endorphin could overcome the action of d-CR14. Nine

groups of animals (5–8 animals per group) were tested. The rats received 3 injections before testing in the small open field. The first injection was saline or dialyzed monoclonal anti-idiotype antibody (d-CR14) diluted 1:10000 with saline. After 10 min saline or desenkephalin-γ-endorphin (0.1, 1.0, 10, 100, 1000 or 10000 ng) was injected. This was followed by an injection of saline or apomorphine (10 ng) after 40 min. The rats treated with antibody received a particular dose of desenkephalin-γ-endorphin and apomorphine. The rats were behaviorally tested 20 min after the last injection.

Experiment 7

Next, the effect of (sub)chronic treatment with dialyzed monoclonal anti-idiotype antibody was tested. Three groups of animals ($n = 10$ per group) were bilaterally injected into the nucleus accumbens twice daily (9.00 a.m. and 4.00 p.m.) for 10 days with dialyzed monoclonal anti-idiotype antibody (d-CR14), the dialyzed control solution (d-control) or saline. The last injection was given on day 10 at 9.00 a.m. On day 11 the animals were tested in the small open field. A second test was performed on day 14.

Experiment 8

To test the function of presynaptically located dopamine receptors, the rats treated (sub)chronically with dialyzed monoclonal anti-idiotype antibody were challenged with a low dose of apomorphine. Two groups of animals ($n = 12$ per group) were bilaterally injected into the nucleus accumbens twice daily (9.00 a.m. and 4.00 p.m.) for 10 days with dialyzed monoclonal anti-idiotype antibody (d-CR14) diluted 1:10 with saline or rat serum diluted 1:10 with saline (control treatment). On day 11 half of the animals of each group were injected with saline and the other half with apomorphine (10 ng). Twenty minutes after this injection the rats were behaviorally tested in the small open field.

Experiment 9

This experiment was designed to test the habituation response of rats treated (sub)chronically with dialyzed monoclonal anti-idiotype antibody. Two groups of animals ($n = 7–9$ per group) were bilaterally injected into the nucleus accumbens twice daily (9.00 a.m. and 4.00 p.m.) for 10 days with dialyzed monoclonal anti-idiotype antibody (d-CR14) diluted 1:10 with saline or rat serum diluted 1:10 with saline (control treatment). On day 11 the rats were tested 5 times in the small open field with an intertrial interval of 20 min.

Experiment 10

To investigate further whether the anti-idiotype antibody displays similar effects as the polyclonal desenkephalin-γ-endorphin antibodies (Van Ree et al., 1982c), an experiment with the passive avoidance test procedure was performed. Two groups of animals ($n =$ 10–13 per group) were bilaterally injected into the nucleus accumbens twice daily (9.00 a.m. and 4.00 p.m.) for 10 days with dialyzed monoclonal anti-idiotype antibody (d-CR14) diluted 1:10 with saline or rat serum diluted 1:10 with saline (control treatment). They were trained for passive avoidance behavior on days 8 and 9 of treatment and were tested for retention on day 10 of treatment.

2.5. Histological evaluation

After the test the animals were decapitated. The brains were removed and fixed in a 4% formaldehyde solution at room temperature for at least 4 days. The position of the cannulae was histologically evaluated according to Pellegrino and Cusham (1967). Data obtained from animals with cannulae outside the nucleus accumbens were discarded from analysis.

2.6. Statistical analysis

The data were analyzed using parametric tests (one- or two-way analysis of variance, and multiple analysis of variance when appropriate) except the data on passive avoidance behavior, which were analyzed using the Mann Whitney *U*-test. Group means and S.E.M. were calculated and presented; for passive avoidance data the median is given.

2.7. Drugs and antibodies

Apomorphine hydrochloride (apomorphine) and Hypnorm (10 mg · ml^{-1} fluanizon, 0.315 mg · ml^{-1} fentanyl citrate) were obtained from OPG, Utrecht, Netherlands and Janssen Pharmaceutica, Tilburg, Netherlands, respectively. Desenkephalin γ-endorphin (β-endorphin-(6–17)) was donated by Organon International, Oss, Netherlands. Apomorphine and desenkephalin-γ-endorphin were dissolved in saline on the day of use.

The polyclonal rabbit antiserum L10 was raised against synthetic γ-endorphin conjugated to bovine thyroglobulin and extensively characterized. The characteristics of this antiserum have been described previously (Sweep et al., 1990). It selectively recognizes the C-terminal region of γ-type endorphins (i.e. the γ-endorphin-(10–17) sequence) and has no affinity for structurally related endorphins, for instance α-endorphin and β-endorphin. Control serum was produced from blood of non-immunized rabbits. The production and characteristics of the oxytocin antibodies have been described before (Liu and Burbach, 1987).

For the monoclonal desenkephalin-γ-endorphin an-

tibody, rats from the Louvain strain were immunized with a keyhole limpet haemocyanin-desenkephalin-γ-endorphin conjugate (Bruning et al., in preparation). After anti-desenkephalin-γ-endorphin antibodies were detected in the serum, spleen cells were fused with SP_2O cells to obtain hybridomas producing anti-desenkephalin-γ-endorphin-antibodies. These were cloned and culture supernatants were screened for desenkephalin-γ-endorphin binding using enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) systems. Selected clones were further characterized with respect to the specificity of the antibodies. Clone CR1B3 produced IgGs that bind γ-endorphin and desenkephalin-γ-endorphin but which display no affinity for α-endorphin or β-endorphin (10000 cpm ^{131}I-labelled peptides). Scatchard plot analysis of [^{125}I]γ-endorphin binding to CR1B3 revealed an affinity constant (K_d) of 2.73 nM. Both γ-endorphin and desenkephalin-γ-endorphin, and at higher concentrations also γ-endorphin-(10–17), concentration dependently inhibited the binding of [^{125}I]γ-endorphin to the antibodies (10000 cpm, binding ca. 25% of total radioactivity). Peptides lacking the γ-endorphin carboxy terminal, like Met-enkephalin, α-endorphin and β-endorphin, did not affect the binding of [^{125}I]γ-endorphin to CR1B3. Next, CR1B3 IgGs were purified from hybridoma culture supernatant on a Sepharose anti-rat IgG immuno-adsorbent column. Rats from the same strain were immunized with the CR1B3 IgG and, after detection of anti-idiotype antibodies in the serum, hybridomas were produced by fusing spleen cells with SP_2O cells, cloned and screened for anti-idiotype antibody production. The anti-idiotype antibodies were screened for their potency to displace radioiodinated γ-endorphin from the CR1B3 antibodies. Two anti-idiotype monoclonal antibodies were selected that showed high displacing activity in these tests. In addition, they did not bind desenkephalin-γ-endorphin, γ-endorphin, α-endorphin or β-endorphin. These monoclonal antibodies, coded CR14 and CR22, were tested for bioactivity. Subsequent experiments were performed with CR14 and dialyzed CR14 (d-CR14). Dialyzed control supernatant (d-control) was produced from SP_2O cultures. Displacement curves of [^{125}I]γ-endorphin binding to CR1B3 (final dilution 1:40000) revealed that the B_{max} decreased with 50% in the presence of about 160 ng CR14. Scatchard analysis indicated a reduction in the B_{max} and no change in the affinity of CR1B3 for the radioligand in the presence of CR14, suggesting an interaction at the level of the idiotype.

3. Results

The position of the tips of cannulae appeared to be in the middle and anterior part of the nucleus accumbens (a representative example is shown in Fig. 1).

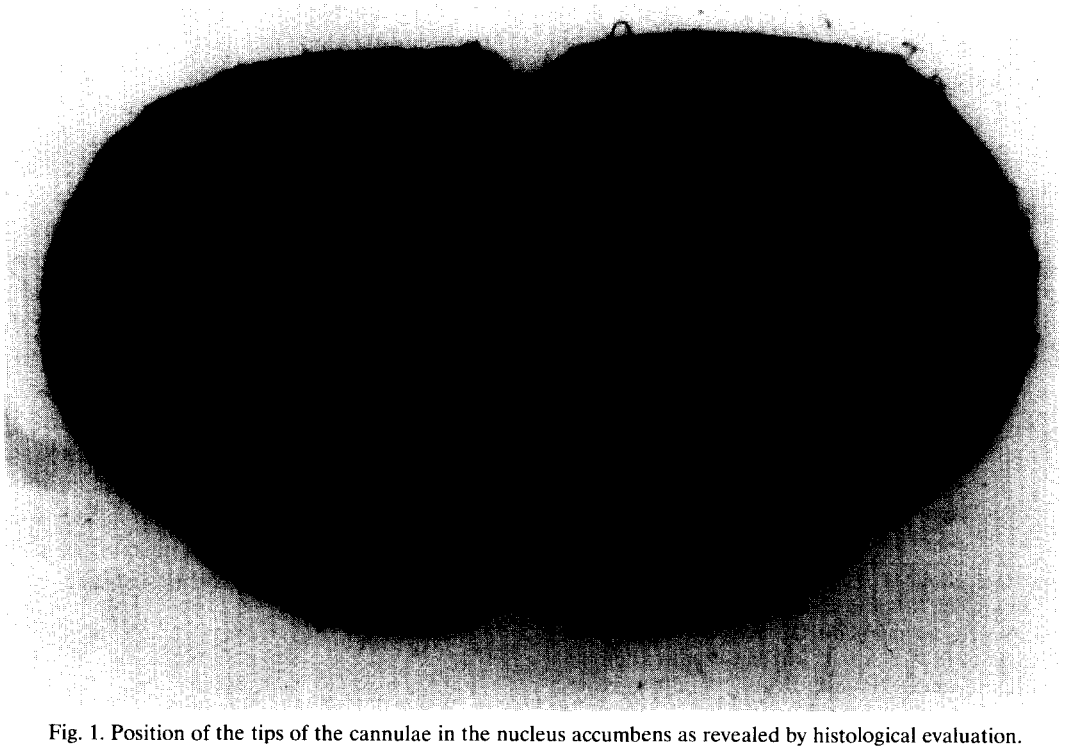

Fig. 1. Position of the tips of the cannulae in the nucleus accumbens as revealed by histological evaluation.

Table 1
The interaction of polyclonal γE-endorphin (γE) antibodies (L10) and oxytocin antibodies after injection into the nucleus accumbens with the desenkephalin-γ-endorphin (DEγE)-induced inhibition of apomorphine-induced hypoactivity

Treatment		Saline, saline [a]	Saline, apomorphine	DEγE, apomorphine	ANOVA
Rabbit serum	1:10 [b]	20.1 ± 0.8 [c] (15)	13.1 ± 0.3 (15) [+]	19.2 ± 0.6 (15) [*]	$F(2,42) = 37.6$ [°]
	1:100	18.9 ± 0.7 (15)	12.7 ± 0.4 (15) [+]	19.7 ± 1.1 (7) [*]	$F(2,34) = 35.3$ [°]
γE antibodies	1:10	19.8 ± 0.9 (8)	14.0 ± 0.6 (9) [+]	13.8 ± 0.5 (6)	$F(2,20) = 25.1$ [°]
	1:100	18.5 ± 0.9 (8)	13.8 ± 0.5 (8) [+]	15.5 ± 0.9 (6)	$F(2,19) = 10.8$ [°]
Oxytocin antibodies	1:10	19.3 ± 1.4 (6)	13.0 ± 0.8 (6) [+]	19.3 ± 1.1 (6) [*]	$F(2,15) = 10.1$ [°]

[a] Two injections: saline or desenkephalin-γ-endorphin (100 pg) and saline or apomorphine (10 ng) at 60 min and 20 min respectively before testing. [b] Injection 70 min before testing with the indicated antibodies diluted 1:10 or 1:100 with saline. [c] Mean ± S.E.M. motor activity score; ( ) number of animals per group. [+] $P < 0.05$, as compared to saline instead of apomorphine treatment (Newman-Keuls). [*] $P < 0.05$, as compared to saline instead of desenkephalin-γ-endorphin treatment (Newman-Keuls). [°] $P < 0.01$.

3.1. Experiment 1

In this experiment the effect of polyclonal γ-endorphin antiserum on the behavioral effect of desenkephalin-γ-endorphin was tested. For control reasons animals were treated with diluted rabbit serum and with diluted oxytocin antiserum. Injection of apomorphine (10 ng) into the nucleus accumbens decreased motor activity, as assessed in the small open field, 20 min after injection. This decrease could be prevented by pretreatment with 100 pg desenkephalin-γ-endorphin (Table 1). Polyclonal γ-endorphin antiserum L10 diluted 1:10 or 1:100 with saline did not interfere with the motor activity of saline- or apomorphine-treated animals, but antagonized the effect of desenkephalin-γ-endorphin. Oxytocin antiserum did not affect the action of desenkephalin-γ-endorphin.

3.2. Experiment 2

The influence of the desenkephalin-γ-endorphin monoclonal antibody on the behavioral effect of desenkephalin-γ-endorphin was tested. The apomorphine-induced hypomotility was antagonized by treatment with desenkephalin-γ-endorphin (Table 2). This antagonizing action was not present in animals treated with the desenkephalin-γ-endorphin monoclonal antibody (CR1B3). Treatment with this antibody interfered with neither the motor activity per se, nor the effect of apomorphine, as assessed with the present test procedure.

Table 2
Interaction between monoclonal desenkephalin-γ-endorphin antibody (CR1B3) and the desenkephalin-γ-endorphin (DEγE)-induced inhibition of the apomorphine-induced hypoactivity after injection into the nucleus accumbens

Treatment [a]			Motor activity score [b] (mean ± S.E.M.)	n
−70 min	−60 min	−20 min		
S	S	S	20.0 ± 0.7	10
S	S	Apo	13.9 ± 1.1 [+]	8
S	DEγE	Apo	20.2 ± 0.5 [°]	8
CR1B3	S	S	19.1 ± 1.5	6
CR1B3	S	Apo	14.8 ± 0.7 [+]	6
CR1B3	DEγE	Apo	15.3 ± 1.4	6

[a] Three injections were given: at −70 min saline (S) or antibody (CR1B3), at −60 min saline (S) or desenkephalin-γ-endorphin (100 pg), at −20 min saline or apomorphine (10 ng). [b] ANOVA on all data: $F(5,38) = 4.3$, $P < 0.01$. [+] $P < 0.05$ as compared to saline instead of apomorphine treatment (Newman-Keuls). [°] $P < 0.05$ as compared to saline instead of desenkephalin-γ-endorphin (Newman-Keuls).

3.3. Experiment 3

Next, the effect of two monoclonal anti-idiotype antibodies (CR14 and CR22) on the behavioral effect of desenkephalin-γ-endorphin was investigated. The apomorphine-induced decrease in motor activity was antagonized by desenkephalin-γ-endorphin (Table 3). The action of desenkephalin-γ-endorphin was inhibited by both monoclonal antibodies. The antibodies did not interfere with the motor activity of saline- or apomorphine-treated animals that did not receive desenkephalin-γ-endorphin.

3.4. Experiment 4

To assess whether material other than proteins present in the monoclonal anti-idiotype antibody preparation was responsible for the inhibition of the desenkephalin-γ-endorphin effect, the effect of treatment with this preparation was compared to that of a dialyzed preparation of this antibody and a control dialyzed preparation. Motor activity was decreased in animals pretreated with monoclonal anti-idiotype antibody and treated with desenkephalin-γ-endorphin and apomorphine compared to that of animals pretreated with saline (Table 4). A similar effect was found with the dialyzed preparation. The control solution (d-control) was not effective in this respect.

3.5. Experiment 5

In order to assess the amount of the dialyzed monoclonal anti-idiotype antibody preparation that could

Table 3
The interaction between monoclonal anti-idiotype antibodies (CR22 and CR14) and the desenkephalin-γ-endorphin (DEγE)-induced inhibition of the apomorphine-induced hypoactivity after injection into the nucleus accumbens

Treatment	Saline, saline [a]	Saline, apomorphine	DEγE, apomorphine	ANOVA
Saline [b]	19.0 ± 0.7 [c] (12)	13.1 ± 0.7 (12) [+]	17.9 ± 0.5 (11) [*]	$F(2,32) = 23.1$ [oo]
CR22	18.0 ± 1.0 (5)	12.7 ± 1.0 (6) [+]	14.2 ± 1.2 (6)	$F(2,14) = 5.7$ [o]
CR14	18.4 ± 1.9 (5)	12.3 ± 0.7 (6) [+]	12.8 ± 1.2 (6)	$F(2,14) = 6.8$ [oo]

[a] Two injections: saline or desenkephalin-γ-endorphin (100 pg) and saline or apomorphine (10 ng) at 60 min and 20 min respectively before testing. [b] Injection 70 min before testing with saline, CR22 or CR14. [c] Mean ± S.E.M. motor activity score. ( ) number of animals per group. [+] $P < 0.05$, as compared to saline instead of apomorphine treatment (Newman-Keuls). [*] $P < 0.05$, as compared to saline instead of desenkephalin-γ-endorphin treatment (Newman-Keuls). [o] $P < 0.02$, [oo] $P < 0.01$.

inhibit the effect of desenkephalin-γ-endorphin, graded dilutions of the antibody preparation were tested. Treatment with the anti-idiotype antibody prevented the action of desenkephalin-γ-endorphin in antagonizing the apomorphine-induced decrease in motor activity (Fig. 2). Even when the antibody was diluted 1:10000 this effect was present. A 10 times lower dilution was not effective in this respect.

3.6. Experiment 6

In this experiment it was tested whether increasing the dose of desenkephalin-γ-endorphin could overcome the action of the dialyzed monoclonal anti-idiotype antibody. The maximal dilution of the anti-idiotype antibody that was effective in experiment 5 was used. It appeared that increasing the dose of desenkephalin-γ-endorphin from 100 pg to 10 μg did not affect the action of the antibody (Fig. 3).

3.7. Experiment 7

This experiment was performed to test the effect of (sub)chronic treatment (twice daily for 10 days) with dialyzed monoclonal anti-idiotype antibody. Treatment with the antibody increased motor activity, assessed one day after the last injection, as compared to treat-

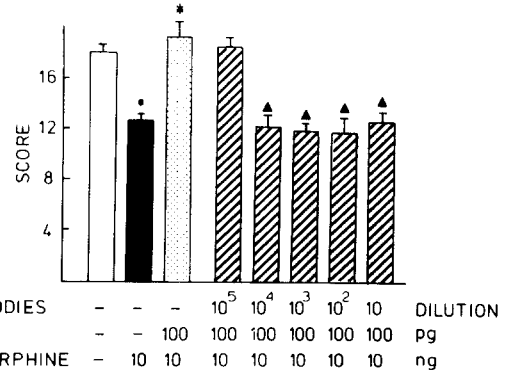

Fig. 2. The interaction between dialyzed monoclonal anti-idiotype antibody and the desenkephalin-γ-endorphin-induced inhibition of the apomorphine-induced decrease in motor activity after injection into the nucleus accumbens. The antibody (d-CR14) was diluted with saline (S) as indicated on the axis. The rats were injected 20, 60 and 70 min before behavioral testing. The mean score (± S.E.M., vertical bars) of motor activity of the different groups is presented. ANOVA on all data: $F(7,60) = 13.8$, $P < 0.001$. [•] $P < 0.05$, as compared to saline instead of apomorphine treatment. [*] $P < 0.05$, as compared to saline instead of desenkephalin-γ-endorphin treatment. [▲] $P < 0.05$, as compared to saline instead of treatment with antibody.

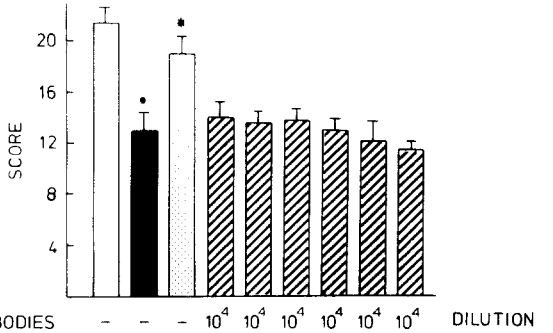

Fig. 3. The interaction between dialyzed monoclonal anti-idiotype desenkephalin-γ-endorphin antibody and the desenkephalin-γ-endorphin-induced inhibition of the apomorphine-induced decrease of motor activity after injection into the nucleus accumbens. The antibody (d-CR14) was 1:10000 diluted with saline (S). The dose of desenkephalin-γ-endorphin varied between 100 pg and 10 μg as indicated on the axis. The rats were injected 20, 60 and 70 min before behavioral testing. The mean score (± S.E.M., vertical bars) of motor activity of the different group is presented. ANOVA on all data $F(8,52) = 7.9$, $P < 0.001$. [•] $P < 0.05$, as compared to saline instead of apomorphine treatment. [*] $P < 0.05$, as compared to saline instead of desenkephalin-γ-endorphin treatment.

Table 4
Effect of treatment with monoclonal anti-idiotype antibody (CR14), dialyzed CR14 (d-CR14), the dialyzed control solution (d-control) or saline on the desenkephalin-γ-endorphin (DEγE)-induced inhibition of the apomorphine-induced hypoactivity after injection into the nucleus accumbens

Treatment [a]			Motor activity score [b] (mean ± S.E.M.)	n
−70 min	−60 min	−20 min		
S	DEγE	Apo	20.1 ± 0.5	8
CR14	DEγE	Apo	13.8 ± 1.2 [*]	6
d-CR14	DEγE	Apo	15.0 ± 1.2 [*]	6
d-control	DEγE	Apo	21.5 ± 1.0	6

[a] Three injections were given: at −70 min saline (S) or antibody or SP_2O, at −60 min desenkephalin-γ-endorphin (100 pg), at −20 min apomorphine (10 ng). [b] ANOVA: $F(3,22) = 5.0$, $P < 0.01$. [*] $P < 0.05$ as compared to saline or d-control instead of antibody (Newman-Keuls).

Table 5
Effect of treatment with dialyzed monoclonal anti-idiotype antibody (d-CR14), the control solution (d-control) or saline, injected into the nucleus accumbens twice daily for 10 days

Treatment	Motor activity score (mean ± S.E.M.)		n
	Day 11	Day 14	
Saline	16.7 ± 0.9	10.1 ± 0.6	10
d-Control	16.7 ± 0.6	11.1 ± 0.7	10
d-CR14	20.4 ± 1.3 *	13.8 ± 1.5 +	10

The rats were behaviorally tested on days 11 and 14 after the start of the experiment. MANOVA: treatment $F(2,27) = 4.9$, $P < 0.02$; repeated testing $F(1,27) = 205.0$, $P < 0.001$; interaction $F(2,27) = 0.6$ n.s. * $P < 0.05$, different from saline and d-control treatment (Newman-Keuls). + $P < 0.05$, different from saline treatment (Newman-Keuls).

ment with saline or d-control (Table 5). The effect of treatment was still present after 4 days when the animals were tested for a second time, although the effect did not reach statistical significance when compared to the effect of the control treatment. The scores during the second testing were lower than those during the first test due to repeated testing in the same environment.

3.8. Experiment 8

In this experiment the function of presynaptically located dopaminergic receptors in the nucleus accumbens was tested after (sub)chronic treatment with dialyzed monoclonal anti-idiotype antibody, using a challenge dose of apomorphine (10 ng). Injection of apomorphine into the accumbens of animals treated for 10 days with rat serum decreased motor activity (Table 6). Such an effect was not obtained in rats treated with anti-idiotype antibody. Even an increase in motor activity was noted upon challenge with apomorphine. An effect of treatment with antibody was also present, i.e. an increase in motor activity.

Table 6
The effect of apomorphine (10 ng) or saline injected into the nucleus accumbens of rats pretreated with rat serum dilution 1:10 with saline or dialyzed monoclonal anti-idiotype antibody (d-CR14), dilution 1:10 with saline, twice daily for 10 days

Pretreatment	Treatment	Motor activity scores (mean ± S.E.M.)	n
Rat serum	Saline	20.5 ± 1.8	6
Rat serum	Apomorphine	16.5 ± 0.7 *	6
d-CR14	Saline	20.8 ± 0.9	6
d-CR14	Apomorphine	24.2 ± 1.5 *	6

On day 11, the rats were tested in the small open field 20 min after the challenge with saline or apomorphine. * $P < 0.05$, as compared to saline instead of apomorphine treatment. ANOVA: pretreatment $F(1,20) = 11.2$, $P < 0.01$; treatment $F(1,20) = 0.1$, n.s.; interaction $F(1,20) = 9.5$, $P < 0.01$.

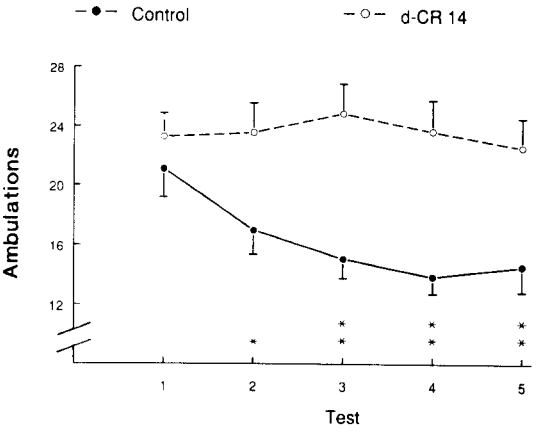

Fig. 4. The habituation response of rats treated twice for 10 days with rat serum, 1:10 diluted with saline (control) or dialyzed monoclonal anti-idiotype antibody (dCR14), 1:10 diluted with saline. One day after termination of treatment the rats were tested 5 times in a small open field for 3 min with an intertrial interval of 20 min. Tested were 7 (rat serum) and 9 (antibodies) animals. ANOVA: treatment $F(1,14) = 10.4$, $P < 0.01$; repeated testing $F(4,56) = 3.4$, $P < 0.02$; interaction $F(4,56) = 3.8$, $P < 0.01$. * $P < 0.05$, ** $P < 0.01$ as compared to control treatment.

3.9. Experiment 9

This experiment was performed to test the habituation response of rats treated (sub)chronically with dialyzed monoclonal anti-idiotype antibody. Habituation was indicated by a decrease in motor activity on repeated testing in the same environment. Habituation was present in rats treated with rat serum, but not in rats treated with anti-idiotype antibody (Fig. 4). The motor activity of the rats treated with antibody was significantly higher than that of control animals.

3.10. Experiment 10

In this experiment passive avoidance behavior was studied in rats treated (sub)chronically with dialyzed monoclonal anti-idiotype antibody injected into the nucleus accumbens. The learning trial took place on day 9 and the retention session on day 10 of treatment. No differences were present between the groups with respect to the latencies during the pre-retention sessions. The latencies of control animals ($n = 13$) during the retention session ranged from 7 to 300 s (median 19 s) and those of rats treated with antibodies ($n = 10$) from 2 to 46 s (median 5 s). This difference ($P < 0.005$, Mann-Whitney U-test) indicates that passive avoidance behavior was attenuated in rats treated with anti-idiotype antibody.

4. Discussion

The present data show that injection of antibodies into the nucleus accumbens can prevent the action of

locally administered desenkephalin-γ-endorphin. This effect was obtained with polyclonal and monoclonal anti-γ-type endorphin antibodies as well as with monoclonal anti-idiotype antibodies. The latter antibodies have been shown to potently compete with desenkephalin-γ-endorphin for its brain binding sites (Ronken et al., 1993). Since these antibodies do not possess affinity for γ-type endorphins (or related peptides), the present effects of these anti-idiotypes may thus pertain to blockade of receptors for γ-type endorphin in the brain. Several control treatments were performed and proved ineffective (i.c. rabbit serum, oxytocin antiserum, control solution for the anti-idiotype antibody), suggesting a rather specific action of the γ-type endorphin antibodies. The anti-idiotype antibody may have high affinity for γ-type endorphin receptors, since its effects on behavior were obtained with low amounts of antibody (high dilution) and even a high dose of desenkephalin-γ-endorphin could not overcome the effect of the antibody. This is consistent with the high affinity of the anti-idiotype monoclonal antibodies CR14 for desenkephalin-γ-endorphin binding sites in the rat brain, as observed with receptor autoradiography of brain slices in vitro (Ronken et al., 1993). Although some of the present test conditions can interfere with the results obtained, e.g. repeated injection into the nucleus accumbens, and the injection of protein material into the brain, it seems that the mechanism underlying the apomorphine and desenkephalin-γ-endorphin effects was hardly affected in the control experiments. Moreover, the desenkephalin-γ-endorphin antibodies did not interfere with the apomorphine action per se.

To further analyze the significance of the putative γ-type endorphin receptors, rats were treated chronically, by nucleus accumbens injection, with anti-idiotype monoclonal antibodies. Such treatment induced long-lasting hypermotility, a decreased habituation response and an attenuated passive avoidance response. Moreover, in such treated animals, local treatment with apomorphine did not elicit a hypomotility response. This latter response is assumed to be due to activation by apomorphine of presynaptically located dopamine receptor sites, leading to a diminished release of dopamine (Costall et al., 1980; Van Ree and Wolterink, 1981; Radhakishun and Van Ree, 1987). Thus, chronic treatment with the anti-idiotype desenkephalin-γ-endorphin antibody may in some way make this receptor system less effective, which could result in a sustained release of dopamine and this could, in turn, explain the observed hypermotility. Biochemical data are not yet available to support this hypothesis. It has been shown, however, that chronic treatment with desenkephalin-γ-endorphin, whereby the γ-type endorphin receptor is stimulated repeatedly, enhances the potency of apomorphine to elicit hypomotility, decreases motor activity and diminishes dopamine release of the nucleus accumbens in vitro and in vivo (Van Ree et al., 1982c; Radhakishun et al., 1994). The present data are in line with the idea that γ-type endorphins are somehow involved in the modulation of the setpoint for feedback regulation of dopaminergic neurons equipped with γ-type endorphin receptor systems and present in the nucleus accumbens (Van Ree et al., 1986). The hypermotility, decreased habituation response and attenuation of passive avoidance behavior observed following chronic treatment with anti-idiotype antibody in the nucleus accumbens have been found before, after identical treatment with polyclonal desenkephalin-γ-endorphin antibodies (Van Ree et al., 1982c). Thus, anti-desenkephalin-γ-endorphin antibody and anti-idiotype desenkephalin-γ-endorphin antibody exert similar effects after both acute and chronic treatment. This validates the present approach of investigating the role of a given peptide receptor system by bioinactivation of the ligand and by blockade of the putative receptor with different antibodies.

It has been hypothesized that a relative deficiency of γ-type endorphins in the brain could result in psychotic symptoms (De Wied, 1978). Thus, rats in which the γ-type endorphin receptors cannot be activated because the ligands are not available or because the receptors are blocked may display disturbances which resemble those observed in schizophrenic patients. Many more studies are needed before definite conclusions can be drawn about such a model system.

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