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Short communication

EFFECT OF DES-TYR^1-γ-ENDORPHIN AND DES-ENKEPHALIN-γ-ENDORPHIN ON ACTIVE AND PASSIVE AVOIDANCE BEHAVIOR OF RATS; A DOSE-RESPONSE RELATIONSHIP STUDY

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The potency of two β-endorphin fragments, des-Tyr^1-γ-endorphin (DTγE, βE-(2-17)) and des-enkephalin-γ-endorphin (DEγE, βE-(6-17)) was compared on extinction of pole-jumping avoidance behavior and on retention of a one-trial step-through passive avoidance procedure. Both peptides facilitated the extinction of pole-jumping avoidance behavior and attenuated passive avoidance behavior. The γ-type endorphins exhibited an inverted U-shaped dose-response curve on passive avoidance behavior but not on extinction of pole-jumping avoidance behavior. DEγE appeared to be approximately three times more potent than DTγE on extinction of pole-jumping avoidance behavior but one hundred times more potent on passive avoidance behavior. It is suggested that DEγE rather than DTγE represents the endogenous neurolepticlike neuropeptide derived from β-endorphin.

γ-type endorphins Active and passive avoidance behavior

1. Introduction

The β-endorphin (βE-(1-31)) fragments α-endorphin (βE-(1-16)) and γ-endorphin (βE-(1-17)) have opposite effects on active and passive avoidance behavior (De Wied et al., 1978a,b). It has been suggested that the behavioral effects of γ-endorphin resemble in some aspects those of neuroleptic drugs (De Wied et al., 1978b). Similar and even more pronounced effects were found with the non-opiate γ-endorphin fragment des-Tyr^1-γ-endorphin (DTγE, βE-(2-17)) (De Wied et al., 1978b). Structure-activity relationship studies with γ-endorphin fragments revealed that des-enkephalin-γ-endorphin (DEγE; βE-(6-17)) which is one of the main metabolites of DTγE (Burbach et al., 1980), is the shortest sequence with neuroleptic-like activity (De Wied et al., 1980).

The present experiments were aimed at comparing the potency of DEγE to that of DTγE using the extinction of pole-jumping avoidance behavior and a one-trial step-through passive avoidance test situation.

2. Materials and methods

2.1. Animals

Male Wistar rats of an inbred strain (CPB-TNO, Zeist, The Netherlands) weighing 140–160 g, were used. The animals were housed 5 per cage at room temperature (20–21°C). All animals had access to commercial food and tap water ad libitum and were kept on a controlled illumination schedule (lights on between 6 a.m. and 7 p.m.).

2.2. Behavioral procedures

2.2.1. Active avoidance behavior

Active avoidance behavior was studied in a pole-jumping avoidance test. Rats were condi-

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tioned to avoid the unconditioned stimulus (US) of an electric footshock (0.25 mA, A.C.) by jumping onto a pole (diameter 1.5 cm) located in the center of the box (30 × 30 × 40 cm). The conditioned stimulus (CS) was a light on top of the cage. The US was applied if an avoidance response had not occurred within 5 s after the onset of the CS. The CS remained on during presentation of the US. Ten acquisition trials were given daily with an average intertrial interval of 60 s. Acquisition training for 4 days was followed by extinction sessions on day 5. In the first extinction session, 10 non-reinforced trials were presented per session in which the CS was terminated immediately after the rat had jumped onto the pole within 5 s (positive response, conditioned avoidance response (CAR)) or after 5 s in the absence of avoidance. Those animals which made 8 or more avoidances at the first extinction session on day 4 were used for further experimentation. The rats received the peptide or saline in a volume of 0.5 ml per rat s.c. immediately after completion of the first extinction session. Two more extinction sessions were run at 2 and 4 h after the first one. The training and extinction sessions were carried out between 9 a.m. and 11 a.m.

2.2.2. Passive avoidance behavior

Animals were trained in a step-through type one-trial learning passive avoidance test. Training was started between 1 p.m. and 6 p.m. The experimental apparatus consisted of an illuminated platform attached to a large, dark compartment equipped with a grid floor. After habituation to the dark compartment (2 min), the rats were placed on the platform and allowed to enter the dark compartment; since rats prefer dark to light, they normally entered within 15 s. On the next day, after three more trials, an unavoidable scrambled footshock (0.25 mA, 2 s) was delivered through the grid floor of the dark compartment (learning trial). The median entrance latencies at the learning trial for the different groups in the various experiments ranged from 3 to 10 s, and the group differences were not significant. Rats were removed from the shock box 10 s after the termination of the shock. Passive avoidance latencies were tested 24 and 48 h after the learning trial; the rat was placed on the platform and the latency to enter the dark compartment was measured up to a maximum of 300 s. Treatments with peptide or saline were given one h before the first retention test.

2.3. Peptides

The peptides des-Tyr^1-γ-endorphin [DTγE, βE-(2-17)] and des-enkephalin-γ-endorphin [DEγE, βE-(6-17)] obtained from Organon International B.V. (Oss, The Netherlands) were dissolved in one drop of 0.01 N HCl then diluted with 0.9% saline (pH: 6.5–6.7). All injections were given s.c. in a volume of 0.5 ml. Control animals received the same volume of the vehicle. Doses between 0.1 ng and 100 ng/rat were used in the active avoidance procedure. Doses ranging from 1 ng to 30 μg/rat were used in the passive avoidance test. With each procedure, the lowest dose which had an effect significantly different from that of saline was used to calculate the potency of the two peptides.

2.4. Statistical analysis

To analyze the data obtained in the pole-jumping avoidance test, the positive responses obtained during the 3rd extinction session of peptide-treated animals were compared with those of saline-treated rats, using Student's t-test. Passive avoidance latencies were analyzed with Mann-Whitney's non-parametric test.

3. Results

Both DTγE and DEγE facilitated the extinction of pole-jumping avoidance behavior and attenuated passive avoidance behavior. In the pole-jumping avoidance procedure, the lowest dose of DEγE which significantly facilitated extinction of pole-jumping avoidance behavior was 1 ng/rat (table 1). To facilitate extinction to a similar degree, a 3 times higher dose (3 ng/rat) of DTγE was needed. The difference in the potencies of DEγE and DTγE was more pronounced in the passive avoidance procedure. Thus, 10 ng/rat of DEγE significantly attenuated passive avoidance behavior at the 24 h (first) and 48 h (second)

TABLE 1

Effect of DEγE and DTγE on the rate of extinction of pole-jumping avoidance behavior in rats.

Treatment	n	Number of avoidances		
		0 [a]	2	4
DEγE				
0.1 ng [b]	6	9.5 ± 0.4 [c]	8.5 ± 0.7	6.6 ± 1.6
0.3 ng	12	9.2 ± 0.3	6.8 ± 2.2	4.5 ± 2.2
1 ng	12	9.2 ± 0.9	4.4 ± 1.5 *	2.6 ± 1.5 **
3 ng	15	8.8 ± 0.4	6.6 ± 0.6	3.4 ± 1.6 *
10 ng	15	9.5 ± 0.3	6.5 ± 1.7	3.3 ± 1.2 *
30 ng	12	9.2 ± 0.5	4.2 ± 1.6 *	3.2 ± 1.5 **
100 ng	6	9.4 ± 0.3	3.8 ± 1.1 **	1.8 ± 0.7 ***
Saline	35	9.6 ± 0.3	8.8 ± 0.5	7.6 ± 0.6
DTγE				
0.3 ng	6	9.6 ± 0.2	7.4 ± 1.4	5.0 ± 1.9
1 ng	12	9.3 ± 0.3	5.1 ± 1.3	3.8 ± 1.5
3 ng	6	9.5 ± 0.3	3.5 ± 1.6 *	1.8 ± 1.1 **
10 ng	6	9.1 ± 0.3	3.8 ± 1.4 **	1.8 ± 1.8 **
30 ng	6	9.1 ± 0.3	3.1 ± 1.3 **	1.6 ± 0.8 **
100 ng	6	9.2 ± 0.2	3.5 ± 1.2 **	1.7 ± 0.9 **
Saline	20	9.3 ± 0.3	8.0 ± 0.8	7.5 ± 0.4

[a] h after injection. [b] Dose per rat s.c. [c] Mean ± S.E. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.001$ (Student's t-test).

TABLE 2

Effect of DEγE and DTγE on retention of the one-trial learning passive avoidance response in rats [a].

Treatment	n	Latency (median s)	
		First retention test (24 h [b])	Second retention test (48 h)
DEγE			
0.001 μg	6	38	28
0.003 μg	6	29	28
0.01 μg	6	19 *	12 *
0.03 μg	6	12 **	13 *
0.1 μg	6	9 ***	3 **
0.3 μg	6	18 **	10 *
1 μg	15	27 *	8 *
3 μg	12	9 ***	21 *
10 μg	15	18 **	49
15 μg	15	16 **	31
30 μg	6	34	48
Saline	25	88	75
DTγE			
0.03 μg	5	62	37
0.1 μg	5	77	39
0.3 μg	5	39	15
1 μg	6	16 **	31
3 μg	5	14 **	7 *
10 μg	6	6 ***	32
15 μg	5	23 *	18
30 μg	6	43	49
Saline	21	85	70

[a] Pre-retention test. [b] h after learning trial. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.002$ (Mann-Whitney U-test).

retention tests (table 2). To obtain a comparable effect, a hundred-fold greater dose (1 μg/rat) of DRγE was needed. However, this dose was not statistically significantly active in the second retention test. No clear dose-response relation was found. Interestingly, a relatively high dose of 30 μg DEγE or DTγE did not significantly affect passive avoidance behavior, indicating a U-shaped dose-response curve.

4. Discussion

The present results confirm earlier findings (De Wied et al., 1978b, 1980) showing that DTγE and DEγE facilitated the extinction of pole-jumping avoidance behavior after peripheral injection, and attenuated passive avoidance behavior after microinjection into the nucleus accumbens (Kovács et al., 1982). Both peptides affected active and passive avoidance behavior in the same way as did the neuroleptic drug haloperidol (De Wied et al., 1978b). DEγE appeared to be three times more potent than DTγE on active avoidance behavior but one hundred times more potent on passive avoidance behavior. In addition, the effect of DEγE on passive avoidance behavior appeared to be longer-lasted. This finding cannot yet be explained but is remarkable because the half-life of DEγE is much shorter than that of DTγE (A. Witter, personal communication).

The extinction of pole-jumping avoidance behavior was facilitated irrespective of whether relatively low or high doses were used. However, doses of DTγE or DEγE much higher than the least effective dose appeared to be inactive in the passive avoidance procedure. This cannot easily be explained. Such U-shaped dose-response curves have been found for the effect of DTγE on the melatonin level in the pineal gland of the rat (Geffard et al., 1981) and with the effects of peptides related to ACTH (Gold and Van Buskirk, 1976).

It is known that performance is related to arousal. A characteristic inverted U-shaped function can describe the relationship: an increase in arousal on the ascending part of the dose-response curve may stimulate the behavior and an increase at the peak or thereafter may have an inhibitory effect (Iversen and Iversen, 1975). In the present experiments this U-shaped function was only found with respect to passive avoidance behavior although higher doses have given the same effect on extinction of pole-jumping avoidance behavior (Le Moal et al., 1980). The reason for this is unknown and may have to do with the type of behavior studied. Interestingly, a similar U-shaped dose-response curve was found for the effects of the ACTH-(4-9) analog (Org 2766) on passive but not on active avoidance behavior (Fekete and De Wied, 1982). Such biphasic effects stress the need for finding the effective dose ranges if the behavioral effects of neuropeptides are to be established.

The increased potency of DEγE as compared to that of DTγE may be due to the absence of the enkephalin moiety [βE-(1-5)] which itself carries information for the inhibition of extinction of pole-jumping avoidance and facilitation of passive avoidance behavior (De Wied et al., 1978a).

Previous studies have shown that DEγE possesses neuroleptic-like activities viz. the effect on active and passive avoidance behavior. In addition, DEγE is also active in various grip tests (De Wied et al., 1980) and in attenuating apomorphine-induced hypolocomotion when injected either s.c. or into the nucleus accumbens (Van Ree et al., 1982). Following their injection into the nucleus accumbens, classical and atypical neuroleptic drugs (Van Ree et al., 1982) have effects similar to those of DEγE. Moreover, clinical studies have shown that DEγE has antipsychotic activities in schizophrenic patients (Verhoeven et al., 1982). These findings led to the hypothesis that reduced bioavailability of γ-type endorphins (as a result of altered synthesis or biotransformation) is an etiological factor in psychopathological states for which neuroleptic drugs are beneficial (De Wied et al., 1978b). The present study of dose-response relationships shows that DEγE is a more potent neuroleptic-like neuropeptide than DTγE and therefore that it rather than DTγE may play a key role as endogenous neuroleptic-like peptide generated from γ-endorphin.

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