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NEUROLEPTIC-LIKE ACTIVITY OF PEPTIDES RELATED TO [DES-TYR^1]γ-ENDORPHIN: STRUCTURE ACTIVITY STUDIES

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Summary

The potency of various fragments of γ-endorphin (β-LPH$_{61-77}$) was compared on their ability to facilitate extinction of pole-jumping avoidance behavior and their effects in two "grip tests" used as measures of neuroleptic-like activity. It appeared that β-LPH$_{66-77}$ is the shortest sequence which shows potencies in the three tests comparable to that of DTγE (β-LPH$_{62-77}$). The activity of β-LPH$_{67-77}$ was less. It is proposed that β-LPH$_{66-77}$ rather than DTγE represents an endogenous neuroleptic-like neuropeptide which may play a key role in psychopathology.

In a previous publication (1) we reported that the β-endorphin fragment γ-endorphin facilitated extinction of active avoidance behavior and attenuated passive avoidance behavior. The influence of γ-endorphin on extinction of pole-jumping avoidance behavior was subsequently confirmed by others (2). The effects were not dependent on opiate receptor activation since the removal of the N-terminal amino acid residue tyrosine which eliminates opiate-like activity, enhanced the influence of γ-endorphin on active and passive avoidance behavior. Further studies indicated that the pharmacological profile of des-1-tyrosine-γ-endorphin (DTγE) in various aspects resembled that of the neuroleptic drug haloperidol although it could not be regarded as a classical neuroleptic compound. Support for antipsychotic activity of DTγE was obtained in clinical studies in a relatively small number of patients (3). In view of these findings we postulated that DTγE or a closely related neuropeptide might be an endogenous neuroleptic with a profile more specific than that of currently used neuroleptic drugs. The present study was undertaken to determine the activity of a number of fragments related to DTγE on extinction of pole-jumping avoidance behavior and in two "grip tests". The results indicate that the sequence β-LPH$_{66-77}$ is the shortest sequence which exhibits neuroleptic-like potencies comparable to that of DTγE.

Materials and Methods

Male Wistar rats of an inbred strain (TNO, Zeist, The Netherlands) were used. They were kept under standard conditions (room temperature 22 + 1° C, light on from 5 a.m. till 7 p.m.) housed in groups of 5 with ad libitum access to food and water.

Behavioral tests

Pole-jumping avoidance behavior

Rats weighing between 130 and 160 g were trained to jump onto a pole with-

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TABLE 1

Effect of Subcutaneous Treatment with Various β-LPH Fragments Related to [Des-Tyr¹]γ-Endorphin (β-LPH62-77) on the Rate of Extinction of Pole-Jumping Avoidance Behavior of Rats.

	Number of avoidances in the 3 extinction sessions			
hours after treatment:	0	2	4	
β-LPH$_{62-77}$				
0.03 µg	8.8 ± 0.3*)	6.5 ± 0.5^{xx}	4.0 ± 1.9^{x}	(4)
0.1 µg	8.8 ± 0.4	2.8 ± 1.0^{xx}	1.2 ± 0.5^{xx}	(5)
saline	9.7 ± 0.4	9.7 ± 0.4	8.7 ± 0.6	(3)
β-LPH$_{65-77}$				
0.1 µg	9.5 ± 0.6	6.3 ± 0.9^{x}	4.5 ± 1.0^{x}	(4)
0.3 µg	9.5 ± 0.4	4.5 ± 0.4^{xx}	2.3 ± 0.9^{x}	(4)
saline	9.8 ± 0.3	9.0 ± 0.4	7.8 ± 0.5	(4)
β-LPH$_{66-77}$				
0.03 µg	8.8 ± 0.5	7.2 ± 1.1	5.8 ± 1.2	(5)
0.1 µg	8.5 ± 0.4	1.7 ± 0.5^{xx}	1.7 ± 0.4^{xx}	(6)
saline	8.6 ± 0.4	8.0 ± 0.5	7.0 ± 0.8	(5)
β-LPH$_{67-77}$				
0.03 µg	9.3 ± 0.4	8.3 ± 0.8	6.2 ± 0.9	(6)
0.1 µg	9.2 ± 0.4	5.0 ± 1.5	2.8 ± 1.4^{x}	(6)
saline	9.6 ± 0.4	7.6 ± 1.5	7.4 ± 1.4	(5)
β-LPH$_{68-77}$				
1 µg	8.8 ± 0.5	6.4 ± 1.5	4.0 ± 1.5	(5)
3 µg	9.3 ± 0.3	7.5 ± 1.0	5.5 ± 2.4	(5)
saline	8.8 ± 0.5	8.0 ± 0.7	7.3 ± 1.0	(4)
β-LPH$_{69-77}$				
1 µg	8.8 ± 0.4	6.2 ± 1.5	6.2 ± 1.5	(5)
3 µg	9.4 ± 0.4	7.4 ± 1.6	6.0 ± 1.5	(5)
saline	9.2 ± 0.5	8.8 ± 0.7	8.0 ± 0.9	(5)
β-LPH$_{70-77}$				
1 µg	8.8 ± 0.5	7.5 ± 1.0	5.5 ± 1.7	(4)
3 µg	9.5 ± 0.4	5.3 ± 2.0	6.0 ± 2.2	(4)
saline	9.7 ± 0.4	8.7 ± 0.4	8.0 ± 0.6	(3)

*) Mean ± Standard Error ( ) Number of rats x $p < 0.05$ xx $p < 0.01$

in 5 sec following presentation of the conditioned stimulus (CS) which was a light on top of the cage (4). Rats which did not jump within 5 sec received scrambled footshocks (0.2 mA) as the unconditioned stimulus (UCS) until the response was made. 10 Trials a day were given in one session with an average intertrial interval of 60 sec. Intervals between trials were 40, 60 and 80 sec which were presented in a random fashion. Rats were trained for 4 days. Extinction was studied the day following acquisition. During the post-acquisition sessions failure to respond within 5 sec to the CS was not followed by the UCS. All rats were given a 10 trial extinction session. Those animals which made 8 or more avoidances were used for further experimentation. These rats received peptide or placebo (saline) immediately after completion of the first extinction session. Two subsequent extinction sessions were run 2 and 4 h later.

Grip tests

Rats weighing between 170 and 190 g were used. First, the front paws of the rats were gently placed onto a pencil that was horizontally positioned approximately 30 cm above the floor of the cage. The grasping response of the animals was assessed using a scale of 0 to 2 (0 = no grasping response: the rat refused to grasp the pencil; 2 = full grasping response: the rat held tightly onto the pencil for a few seconds without releasing it even when the pencil was moved up and down in the air; sometimes the animal tried to lift up its body). The second test was performed using a horizontally positioned rod with a diameter of 9.0 mm and placed approximately 23 cm above the floor of the cage. The rats were gently placed with their front paws onto the rod and the time (in tenth of seconds) was measured that the animals hang suspended above the floor. This test was performed twice in succession; the results of the second test-trial were used only.

Animals were first tested in the two grip tests and immediately thereafter they were injected subcutaneously with 50 µg of the respective peptide dissolved in saline or placebo. Testing was performed again at 30 and 120 min after injection. Between testing the animals were housed in groups of 5 animals per cage in a sound-attenuated room.

Peptides

β-LPH$_{62-77}$ (DTγE); β-LPH$_{65-77}$; β-LPH$_{66-77}$; β-LPH$_{67-77}$; β-LPH$_{68-77}$; β-LPH$_{69-77}$; β-LPH$_{70-77}$. Peptides were prepared by the classical approach of fragment condensation and analysed among others by high performance liquid chromatography (Greven et al., in preparation). The purity of the peptides appeared to be 95-99%.

Statistical analysis

The data obtained in the pole-jump avoidance test were analysed by comparing the responses during the 2nd or 3rd extinction session of peptide treated animals with those of placebo treated rats using the Student t-test. Similar testing was performed on the data of the grip test using the rod. The X^2-test for a 2 x 3 contingency table was used for calculating statistical differences between placebo and peptide treated animals in the grip test using the pencil.

Results

Effects of the various β-endorphin fragments on extinction of pole-jumping avoidance behavior as compared to DTγE are summarized in Table 1. Although β-LPH$_{65-77}$ seemed to be somewhat less potent than DTγE, β-LPH$_{66-77}$ appeared to be as potent. Administration of 0.1 µg of β-LPH$_{66-77}$ had the same effect on extinction as 0.1 µg of DTγE while 0.3 µg of β-LPH$_{65-77}$ was needed to achieve maximal extinction i.e. less than a mean of 3 positive responses in the 3rd extinction session. The peptide β-LPH$_{67-77}$ was somewhat less active since the dose of 0.1 µg induced a significant effect in the 3rd extinction session only

The activity markedly declined when N-terminal amino acids were successively removed from β-LPH$_{67-77}$. At the 2nd and 3rd extinction session no significant difference was found between peptide and saline treatment with respect to β-LPH$_{68-77}$, β-LPH$_{69-77}$ and β-LPH$_{70-77}$.

Effects of the various peptides in the two "grip tests" are summarized in table 2. As compared to DTγE the effects of β-LPH$_{65-77}$ and β-LPH$_{66-77}$ were of the same magnitude in the "pencil-grip test". The responses of β-LPH$_{65-77}$ were less significant in the "rod-grip test". The sequence β-LPH$_{67-77}$ was not active in the "pencil-grip test" in the fixed dose used, and showed only a significant response in the "rod-grip test" at the 30 min interval but not after 120 min. Shorter fragments were not active in this respect. It is of interest to note the similarity in potency of the various fragments in the avoidance test and the "grip tests".

TABLE 2

Effect of Various β-LPH Fragments Related to (Des-Tyr^1)γ-Endorphin (β-LPH$_{62-77}$) in Two "Grip Tests"

	test using pencil[1]			test using rod[2]		
min after injection	before	30'	120'	before	30'	120'
saline[3]	0.04	0.08	0.04	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1
β-LPH$_{62-77}$	0.08	0.67*	0.92**	0.6 ± 0.1	1.6 ± 0.4*	2.0 ± 0.1***
β-LPH$_{65-77}$	0.00	0.46	0.83**	0.3 ± 0.1	1.2 ± 0.3*	1.7 ± 0.4*
β-LPH$_{66-77}$	0.00	0.38	0.67**	0.4 ± 0.1	1.1 ± 0.1**	2.2 ± 0.4**
β-LPH$_{67-77}$	0.04	0.24	0.50	0.4 ± 0.1	0.9 ± 0.1**	1.2 ± 0.2
β-LPH$_{68-77}$	0.00	0.16	0.54	0.6 ± 0.1	0.8 ± 0.2	1.0 ± 0.3
β-LPH$_{69-77}$	0.08	0.08	0.16	0.4 ± 0.1	0.8 ± 0.2	1.0 ± 0.3
β-LPH$_{70-77}$	0.00	0.08	0.08	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

1) data are expressed as proportion of maximal performance = 1.00.

2) Mean (± SEM) of the time (seconds) that the animals hung suspended above the floor.

3) Groups of 6 animals were subcutaneously injected with placebo (0.5 ml) or 50 µg of the respective peptides.

Discussion

The present results indicate that β-LPH$_{66-77}$ is the shortest γ-endorphin fragment with activities quantitatively comparable to DTγE with respect to facilitation of extinction of pole-jumping avoidance behavior and activity in two "grip tests". There was a striking similarity in structure activity relationship of the various γ-endorphin compounds on facilitation of extinction of pole-jumping avoidance behavior and in the "grip tests". This suggests that the two systems measure aspects of a common underlying mechanism characteristic for neuroleptic activity (1). Although the sequence β-LPH$_{67-77}$ still exhibited significant activity in the avoidance test and one of the "grip

tests", it is clear that the complete spectrum of neuroleptic-like activity of DTγE is limited to the sequence β-LPH66-77. Accordingly, not only the N-terminal amino acid residue tyrosine can be removed but the whole enkephalin sequence (β-LPH61-65) which in itself carries information for inhibition of extinction of pole-jumping avoidance behavior (5). Although DTγE did not displace neuroleptics from their binding sites in various brain areas of the rat as measured in vitro (6,7) it was recently found that DTγE administered subcutaneously decreased in vivo ^{3}H-spiperone binding particularly in the corpus striatum and nucleus accumbens areas of rat brain (8). In view of this the authors suggested that DTγE may act either by releasing dopamine, by altering the conformation of neuroleptic binding sites or that DTγE may exert its neuroleptic-like effects through an active metabolite. They tentatively ascribed β-LPH66-77 as the principal metabolite of DTγE when incubated with rat brain homogenate. Moreover, we have preliminary data indicating that β-LPH66-77 interferes with apomorphine-induced changes in locomotor activity. The fact that in the behavioral studies presented here β-LPH66-77 is as active as DTγE on extinction of pole-jumping avoidance behavior and in the "grip tests" suggests that this sequence represents an endogenous neuroleptic-like neuropeptide. If this were demonstrated by in vitro interaction with neuroleptic binding sites, a reduced availability of β-LPH66-77 rather than that of DTγE may be an aetiological factor in psychopathology.

References

1. D. DE WIED, G.L. KOVACS, B. BOHUS, J.M. VAN REE and H.M. GREVEN, Eur. J. Pharmacol. 49, 427-436 (1978).
2. M. LE MOAL, G.F. KOOB and F.E. BLOOM, Life Sci. 24, 1631-1636 (1979).
3. W.M.A. VERHOEVEN, H.M. VAN PRAAG, J.M. VAN REE and D. DE WIED, Arch. Gen. Psychiatry 36, 294-298 (1979).
4. TJ.B. VAN WIMERSMA GREIDANUS and D. DE WIED, Neuroendocrinology 7, 291-301 (1971).
5. D. DE WIED, B. BOHUS, J.M. VAN REE and I. URBAN, J. Pharmacol. Exp. Ther. 204, 570-580 (1978).
6. J.M. VAN REE, A. WITTER and J.E. LEYSEN, Eur. J. Pharmacol. 52, 411-413 (1978).
7. N.W. PEDIGO, N.C. LING, T.D. REISINE and H.I. YAMAMURA, Life Sci. 24, 1645-1649 (1979).
8. N.W. PEDIGO, T. SCHALLERT, D.H. OVERSTREET, N.C. LING, P. RAGAN, T.D. REISINE and H.I. YAMAMURA, Eur. J. Pharmacol. 60, 359-364 (1979).