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NEUROPEPTIDES AND SOCIAL BEHAVIOR OF RATS TESTED IN DYADIC ENCOUNTERS

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ABSTRACT

The effects of various neuropeptides on social behavior was studied in a test procedure in which 7-day isolated animals were tested together with non-isolated partners in dyadic encounters. The short-term isolation procedure increased the frequency and duration of social activities of the rats, but hardly affected non-social explorative behaviors of the animals. Systemic injection of certain neuropeptides, i.c. prolyl-leucyl-glycinamide (PLG), thyrotropin releasing hormone (TRH) and the $ACTH_{4-9}$ analog ORG 2766, reversed the isolation-induced increase in social activity, similarly as previcusly observed with antidepressant drugs. Subcutaneous treatment with β-endorphin, α-endorphin and des-Tyr-γ-endorphin increased social interactions in 7-day isolated animals. β-Endorphin enhanced social behavior of non-isolated rats as well, whereas γ-MSH decreased the social interactions of these animals. Both peptides affected especially social contact behavior. The potent action of β-endorphin suggests that this peptide and opioid systems may play a physiological role in social behavior. It is proposed that a possible functional antagonism between ACTH-like peptides, especially γ-MSH, and β-endorphin may operate in social behavior. The action of the peptides may be rather specific for social behavior, since none of the neuropeptides affected non-social explorative behaviors of the rats during the social interaction test.

INTRODUCTION

In animal models as well as in clinical studies, the effectiveness of psycho-active compounds may strongly be influenced by the experimental set up. Thus, environmental conditions, social setting and previous experiences are capable of modifying responses to these compounds. According to Valzelli and Bernasconi (1) it is more effective to investigate the properties of psycho-

active compounds in animals showing behavioral alterations than in "normal" laboratory animals. They postulated that this would reveal information more closely linked to the clinical profile of the compounds.

Alterations in social behavior of rats can be evoked by isolation. Prolonged socio-environmental deprivation has been shown to produce various types of disturbed behavior like muricide and hyperlocomotion (2,3). Recently we reported that short-term isolation increases socio-explorative behavior in rats when tested in pairs (4). This increase in social interactions in isolated rats is normalized to the level of non-isolated controls by treatment with antidepressants, but not by that with other psychopharmacological drugs (5). Thus, this behavioral procedure may be useful for predicting antidepressant activities of substances.

The expression of social behavior depends on environmental conditions as well as on internal factors (6). Candidates for these internal factors are neuropeptides, because of their proposed role in behavioral adaptation (7). We have therefore investigated the effectiveness of various neuropeptides to change social behavior of non-isolated rats as well as to affect the increased social interactions due to short-term social deprivation. Different peptide fragments derived from pro-opiomelanocortin and some peptides related to posterior pituitary and hypothalamic hormones were tested. Especially the influence of neuropeptides with reported antidepressant properties (8,9,10,11), e.g. prolyl-leucyl-glycinamide (PLG), thyreotropin releasing hormone (TRH) and the $ACTH_{4-9}$ analog ORG 2766 was analysed in detail. Non social explorative behaviors (ambulation, rearing) were simultaneously recorded, to establish whether an influence on social behavior is exerted specifically or through a general sedative effect.

METHODS

Animals

Male Wistar rats, weighing between 160 and 180 grams on the start of the 7-day isolation period were used. The rats were bred from our own stock and housed in wire cages in groups of approximately 20 animals per cage. Seven days prior to experimentation, the rats were either individually housed (I, in this article referred to as isolated) or in groups of 5 rats per cage (S, socially housed, referred to as non-isolated). Now, home cages were of plastic, 22x13 and 26x40 cms respectively. During the isolation period, cages of isolated and non-isolated animals were in the same room, so the isolated animals could hear and smell their conspecifics, but did not have physical contact with them. During this period, the animals were kept in a temperature-controlled environment (25±1 °C), with a regular day/night cycle (20-100 Lux; lights on 7.00 a.m. to 7.00 p.m.). Standard food and tap water were available ad. lib. Animals were used only once.

Experimental procedure

Pairs of one non-isolated (S) and one isolated (I) animal were tested for social interactions. The rats were selected in such a way, that at testing the S- and I-animal of a pair did not differ

in body weight by more than 10 g. Rats were subcutaneously injected with saline (0.5ml) or with graded doses of various peptides 60 minutes before the test. In each experiment 3 different groups of pairs were tested: 1) both, the S- and the I-rat received placebo (referred to as control rats), 2) the S-rat received saline and the I-rat the peptide and 3) the S-rat received the peptide and the I-rat the saline. One hour after injection, a pair of rats was placed in the observation cage and the behavior of the rats was recorded on video (Sony U-matic) for the next 10 minutes and later analysed off line. This analysis was performed blindly. The different treatment groups were tested in a random order between 9.00 a.m. and 1.00 p.m. Frequencies and the duration of occurrence of the following social interactions were scored: exploration of the partner (sniffing or licking any part of the body of the partner), anogenital investigation (sniffing or licking the anogenital area of the partner), crawl-over/mount (the rat climbs on or over the other rat), social grooming (mutual grooming of one animal by another), approach/follow (walking or running in the direction of the other rat), fighting (all aggressive behaviors towards the partner), kicking (kicking backwards at the conspecific with one or both hind legs) and biting (fast snapping and leaving off or holding on while jerking). Of the last two items, frequencies were measured only. For further analysis the items exploration of the partner and anogenital investigation have been combined into one item social explorative behavior; crawl-over/mount and social grooming into contact behavior; and fighting, kicking and biting into aggressive behavior. Because differences in social activity between the first and second part of the test period have previously been observed (12), the behavioral analysis of the 10 minutes test period was divided into two periods of 5 minutes. For non-social explorative behaviors, ambulation (number of floor units crossed), rearing wall (standing on hind legs against the wall), rearing middle (standing on hind legs in the middle of the cage) and selfgrooming were scored. A detailed description of the analysing procedure and of the behavioral items is given elsewhere (4,5).

Peptides

Peptides were dissolved in one drop of 10^{-5} N HCl, and subsequently diluted with 0.9% saline (pH 6.5-6.7) and subcutaneously injected in an amount of 0.5 ml per animal. The following peptides were used: $ACTH_{1-24}$ (H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-OH); $ACTH_{4-10}$ (H-Met-Glu-His-Phe-Arg-Trp-Gly-OH); (D-Phe^{7})$ACTH_{4-10}$ (D-Phenylalanine substituted for L-Phenylalanine in position 7); the $ACTH_{4-9}$ analog ORG 2766 (H-Met/O_2/-Glu-His-Phe-D-Lys-Phe-OH); α-MSH (Ac-Ser-Tyr-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2); γ-MSH (H-Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly-OH); human β-endorphin (H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH); β-endorphin 1-16(α-endorphin; αE); β-endorphin 2-16(des-Tyr^{1}-α-endorphin;DTαE); β-endorphin 2-17 (des-Tyr^{1}-γ-endorphin; DTγE); βendorphin 2-9; TRH (thyrotropin releasing hormone; p-Glu-His-Pro-NH_2); PLG (Pro-Leu-Gly-NH_2); DG-AVP (des-Glycinamide^{9}-(Arg^{8})-vasopressin (H-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-OH) and Oxytocin (H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2).

TABLE I

BEHAVIORAL ITEMS AS OBSERVED IN SOCIAL INTERACTION TESTS IN WHICH 7-DAY ISOLATED RATS (I) WERE TESTED TOGETHER WITH NON-ISOLATED PARTNERS (S).

	FREQUENCY			DURATION (sec)		
Behavioral item:	S -	I	Δ I-S	S -	I	Δ I-S
Exploration partner:	29.0±2.0 -	44.0±2.0	p<0.01	25.8±1.6 -	40.6±2.0	p<0.01
Anogenital investigation:	21.8±1.6 -	37.4±2.8	p<0.01	21.2±1.7 -	40.8±3.3	p<0.01
Crawl over/Mount:	4.1±0.6 -	11.3±1.2	p<0.01	3.0±0.5 -	9.8±1.4	p<0.01
Social grooming:	5.2±0.7 -	10.8±1.4	p<0.01	9.0±2.3 -	14.6±2.4	p>0.05
Approach/Follow:	11.6±1.1 -	20.0±1.3	p<0.01	9.8±0.7 -	22.0±1.6	p<0.01
Biting*:	0.5 -	2	p<0.01			
Kicking*:	2 -	2	p>0.05			
Fighting*:	1 -	2	p>0.05	0.9±0.3 -	2.2±0.7	p>0.05
Total number of social interactions:	73.5±3.3 -	130.3±6.5	p<0.01	69.7±4.0 -	129.9±5.6	p<0.01
Ambulations:	352.7±11.3 -	341.6±8.5	p>0.05			
Rearing wall:	56.2±2.8 -	49.6±3.7	p>0.05			
Rearing field:	33.6±2.4 -	24.9±1.7	p<0.01			
Selfgrooming:	10.3±1.5 -	4.5±0.7	p<0.01			

Data of 35 pairs are given as the mean (±S.E.M.) or median (*) frequency or duration that a certain item occurred in the 10 minute session. Statistical analysis was performed using Student's t-test or Mann Whitney U-test. Δ= difference between I- and S-animals.

Statistics

For most of the variables a Wilk-Shapiro test for normality on the data indicated that the underlying population did not deviate from a normal distribution. Here parametric statistical methods, otherwise non-parametric statistical tests were used. Data of the peptides were analysed with two-way analyses of variance tests (ANOVA) for dose levels and housing conditions of simultaneously tested groups. When p values were <0.05, Student's t-tests (two-tailed) were performed on the individual dose groups and their controls. For comparing data observed from the same animal, paired t-tests (normally distributed) or Wilcoxon sign tests (not normally distributed) were used.

RESULTS

Body weights were not affected by the 7-day isolation procedure. During this time period a mean increase in body weight of 37.3 ± 1.3 g in non-isolated rats was observed (161.8 ± 1.5 on day 1; 205.5 ± 2.1 on day 8, Mean $\pm$ S.E.M., n=35), versus an increase in isolated rats of 35.5 ± 1.2 g (165.9 ± 1.3 and 201.4 ± 1.8 on day 1 and 8 respectively, n=35). Mean frequency and duration of the measured behavioral items of 35 pairs of placebo treated animals in the 10-minute test session are presented in Table I. The most frequently observed items in the non-isolated animals were exploration partner (39% of total) and anogenital investigation (29% of total). Thus, social explorative behavior accounts for 68% of total number of social interactions. Less frequently occurred contact behavior (12% of total, this item includes crawl-over/mount and social grooming) and the item approach/follow (15% of total). Aggressive behavior, which includes biting, kicking and fighting, formed only approximately 5% of total frequency of social interactions. A similar distribution over the various items was found with respect to the duration of the social activities. Thus, in general the social activities were amicable social behaviors while aggressive or sexual behavior hardly occurred. No differences in frequency of social explorative behavior, contact behavior, approach/follow and total number of social interactions were observed between the first and second period of 5 minutes in non-isolated animals. Aggressive behavior was significantly ($p<0.03$, sign-test) increased in the second half of the test period. With respect to the time spent on the different social activities, no difference was found between the first and second half of the test period.

In the 7-day isolated animals, social explorative behavior accounted for 63%, contact behavior for 17%, approach/follow for 15% and aggressive behavior for 5% of the total number of social interactions per 10 minutes. A similar distribution was found for the duration of social contacts (Table I). Aggressive behavior ($p<0.007$, sign-test) was significantly increased in the second half of the test period. No differences between the first and second period of 5 minutes were observed in any of the other social activities, neither in frequency nor in duration.

Comparing the social activities in 10 minutes in isolated animals with those of non-isolated rats, frequencies of all social activities except kicking were increased in the isolated animals.

TABLE II

EFFECTS OF NEUROPEPTIDES ON TOTAL NUMBER OF SOCIAL INTERACTIONS IN NON-ISOLATED AND 7-DAY ISOLATED ANIMALS.

TREATMENT		ISOLATED RATS		NON-ISOLATED RATS	
Peptide	Dose µg/kg	n	% of controls	n	% of controls
Placebo (controls)			100		100*
ACTH 1-24	50	6	114 ± 5	6	102 ±10
ACTH 4-10	50	6	93 ±10	12	83 ± 8
ACTH 4-10 (D-Phe)	50	6	107 ± 7	6	88 ±13
α-MSH	50	6	93 ± 6	6	102 ±14
γ-MSH	50	6	98 ± 7	12	74 ± 8 ↓ *
ORG 2766	0.01	6	106 ± 8	6	118 ±14
ORG 2766	0.05	7	78 ± 7 ↓**		n.d.
ORG 2766	0.1	6	68 ± 8 ↓**	6	108 ±16
ORG 2766	1	6	97 ±14	6	97 ±11
ORG 2766	50	6	91 ± 8	6	70 ±12 ↓ *
TRH	0.1	6	91 ± 6		n.d.
TRH	1	5	84 ± 6 ↓ *		n.d.
TRH	10	5	80 ± 5 ↓**	6	114 ±15
TRH	100	5	75 ± 9 ↓**	6	105 ±12
PLG	0.1	5	72 ± 5 ↓ *		n.d.
PLG	1	5	74 ± 7 ↓**		n.d.
PLG	10	5	73 ± 5 ↓**	6	94 ±13
PLG	100	5	72 ± 5 ↓**	6	85 ±11
β-ENDORPHIN	0.001	5	109 ± 8		n.d.
β-ENDORPHIN	0.01	5	138 ± 8 ↑**		n.d.
β-ENDORPHIN	0.1	12	130 ± 9 ↑**		n.d.
β-ENDORPHIN	1	12	124 ± 5 ↑**	6	115 ± 8
β-ENDORPHIN	10	13	136 ±10 ↑**	12	128 ± 6 ↑**
β-ENDORPHIN 1-16 (α-E)	250	7	130 ± 7 ↑ *	7	104 ±14
β-ENDORPHIN 2-16(DTαE)	250	7	92 ± 9	7	99 ± 9
β-ENDORPHIN 2-9	250	6	110 ± 7	7	93 ± 9
β-ENDORPHIN 2-17(DTγE)	5	18	128 ± 8 ↑**	18	105 ±21
DG-AVP	50	6	123 ± 9	6	108 ±15
OXYTOCIN	50	6	100 ±12	6	116 ± 8

Rats were subcutaneously treated 1 hour before the 10 min. social interaction test. The partners of the peptide treated animals received placebo. Data are mean (± S.E.M.) frequencies and expressed as percentages of the corresponding animals in simultaneously tested I-placebo - S-placebo pairs. Only data of the peptide treated rats are presented.

** The social activities of placebo treated, non-isolated rats appeared to be 56% of that of placebo treated, isolated animals (96 pairs of control rats).*

n = number of pairs tested.

n.d. = not determined.

↑ = significant increase as compared to simultaneously tested controls.

↓ = significant decrease as compared to simultaneously tested controls.

** Differenet from simultaneously tested, placebo treated control rats, (* $p<0.05$; ** $p<0.01$; Student's t-tests, two-tailed.*

The higher frequency was accompanied by an increase in time spent in social contact, although among the individual items the durations of social grooming and fighting were not significantly higher in the isolated animals. The differences in social activities between isolated and non-isolated animals were observed in the first as well as in the second half of the test period. With respect to the non-social activities per 10 minutes, rearing field and selfgrooming were significantly decreased in the isolated animals; rearing field being decreased in the first period of 5 minutes, whereas selfgrooming was in the second period.

The effects of different neuropeptides on the frequency of social interactions are summarized in Table II. Data are given as percentage of simultaneously tested placebo-treated control rats (see methods). ACTH-related peptides tested in a dose of 50 µg/kg did not significantly affect the total number of social interactions, neither in the isolated nor in the non-isolated ones, with the exception of γ-MSH and ORG 2766. Both these peptides significantly decreased social interactions, but in the non-isolated rats only. A tendency of a similar effect was observed with ACTH-4-10. Lower doses of ORG 2766 (0.05 and 0.1 µg/kg), and also graded doses of TRH (1.0-100 µg/kg) and PLG (0.1-100 µg/kg) significantly decreased the total number of social interactions in the isolated animals, but not in the non-isolated ones.

In contrast, low doses (0.01-10 µg/kg) of β-endorphin significantly increased social interactions of the isolated rats (see also 13). A similar increase was found following treatment of isolated rats with 250 µg/kg of α-endorphin (β-endorphin 1-16). With the exception of the 10 µg/kg dose of β-endorphin, these effects were not observed in the non-isolated animals. A slight, but statistically significant increase in social interactions was found with one of four tested doses of DTγE (5 µg/kg), but a lower (1 µg/kg) and two higher doses (25 and 125 µg/kg) did not affect the total number of social interactions. β-Endorphin 2-9 (250 µg/kg), β-endorphin 2-16 (DTαE; 5 and 250 µg/kg) and DG-AVP and oxytocin (50 µg/kg) did not significantly change the frequency of social interactions, neither in the isolated nor in the non-isolated animals. In none of the tested rats a change in social interactions was observed in the placebo-treated test partners of the peptide-treated rats.

Further analysis revealed that in general ORG 2766, PLG and TRH decreased all social interactions of the isolated animals. Thus, social explorative behavior (= exploration partner + anogenital investigation), contact behavior (= crawl-over/mount + social grooming) and the item approach/follow were attenuated by peptide treatment. Aggressive behavior was not analysed in detail, since this behavior occurred with a low frequency and was susceptable to change among the various experiments. Most consistently decreased by peptide treatment was social explorative behavior. This item accounted for more than 60% of the total frequency of social interactions and was increased with 60% by the isolation procedure (see table I). The effects of the peptides were most pronounced during the first 5 minutes of the test period. The decrease in frequencies of social interactions was accompanied by a similar decrease in the time spent on social activity. No effect of these peptides was observed with respect to the non social activities,

TABLE III

EFFECT OF NEUROPEPTIDES ON AMBULATION AND REARING IN THE SOCIAL INTERACTION TEST.

TREATMENT		ISOLATED ANIMALS		
Peptide	Dose µg/kg	Ambulation	Rearing (wall + field)	n
ORG 2766	0.01	94 ± 6	105 ± 11	6
	0.05	101 ± 1	88 ± 12	7
	0.1	100 ± 7	115 ± 12	6
	1	111 ± 4	102 ± 9	6
	50	110 ± 7	114 ± 12	6
TRH	0.1	107 ± 8	105 ± 6	6
	1	102 ± 5	110 ± 8	5
	10	100 ± 6	114 ± 6	5
	100	102 ± 9	97 ± 6	5
PLG	0.1	94 ± 6	95 ± 9	5
	1	94 ± 8	106 ± 8	5
	10	88 ± 5	94 ± 8	5
	100	99 ± 6	99 ± 12	5
β-ENDORPHIN	0.001	82 ± 4 ↓	90 ± 11	5
	0.01	100 ± 5	104 ± 9	5
	0.1	102 ± 7	101 ± 7	12
	1	97 ± 7	79 ± 6	12
	10	94 ± 6	88 ± 7	13
		NON-ISOLATED ANIMALS		
ACTH 4-10		92 ± 12	110 ± 12	6
ORG 2766		115 ± 6	108 ± 11	6
γ-MSH		99 ± 7	118 ± 13	6
β-ENDORPHIN	1	92 ± 12	100 ± 12	6
	10	98 ± 6	105 ± 6	12

Rats were subcutaneously treated 1 hour before the 10 min. social interaction test. The partners of peptide treated rats received saline. Data are presented as mean (± S.E.M.) frequencies and expressed as percentage of simultaneously tested placebo treated control animals. Statistical analysis was performed using Student's t-tests (two tailed). n = number of pairs tested. ↓ = significant decrease as compared to simultaneously tested controls ($p<0.001$).

neither per 10 minutes (Table III), nor in the first or in the second half of the test session.

The increase in social interactions by β-endorphin in isolated and non-isolated rats was due to an increase in contact behavior in particular (13). Social explorative behavior and the item approach/follow was hardly changed by peptide treatment. The increase in frequency of social interactions by β-endorphin was accompanied by an increase in the duration of social behavior, but only time spent in contact behavior was significantly increased. β-Endorphin had no effect on the non-social activities in isolated animals during the social interaction test (Table III).

Frequency of social interactions in non-isolated rats was decreased by 50 µg/kg of γ-MSH and ORG 2766 (Table II). A tendency towards a decrease of social interactions was found with ACTH 4-10 (50 µg/kg). The decrease in frequencies of social interactions by ORG 2766 was due to a (non significant) decrease in social explorative behavior in the first and the second half of the test session. The influence of ACTH 4-10 and γ-MSH was most consistent on the item contact behavior and particularly obvious during the second half of the test session (66 ± 17 and 52 ± 16% of controls for ACTH 4-10 and γ-MSH respectively).
The duration of social interactions in the ACTH 4-10- and γ-MSH-treated rats was however not changed. As a consequence, a significant increase in the ratio time/frequency (=indication for mean duration of contacts) of contact behavior in the second part of the test session was found (placebo: 100 ± 4%; ACTH 4-10: 118 ± 5%, $p<0.01$ and γ-MSH: 124 ± 5%, $p<0.01$).
Non-social activities of non-isolated animals during the interaction test were not affected by treatment with ACTH 4-10, ORG 2766 and γ-MSH (Table III).

DISCUSSION

Consistent with previous observations (4,5), short term social isolation increases the frequency of social interactions of rats, without affecting the locomotor activity of these animals. This increase in social activity is not caused by a shorter duration of the individual contacts, because the duration of these contacts increases simultaneously with the frequency of social interactions. Also others have reported an increase in time spent in social contact after social deprivation (e.g. 14,15,16). Thus, social deprivation may lead to an increase of the motivation to perform social behavior. The enhancement in social behavior seems to be rather specific and can not simply be accounted for by a general increase in activity. In fact, the isolation procedure did not change locomotor activity (ambulation) of the rats, while explorative behavior (rearing field) and skin-care behavior (selfgrooming) were decreased rather than increased.

Little is known as yet about possible biochemical processes underlying the change in social behavior induced by short-term isolation. Several investigators have reported changes in brain-amine levels and turn-over rates in different brain areas after more prolonged isolation (17,18,19). However, no clear correlation between these biochemical modifications and the changes in be-

havior could be demonstrated. Schenk et al. (1982) reported that isolation decreased the number of opiate receptors in rat brain. Moreover, short-term isolated rats are more sensitive to pain (21, 22). Recently, brain opioid systems have been implicated in social emotional processes (23). Thus, a change in endogenous opioid systems may be involved in the increase in gregariousness by short-term social isolation.

The present study shows that the increase in social activity, induced by short-term isolation, can selectively be blocked by treatment with the tripeptides PLG and TRH and with the ACTH 4-9 analog ORG 2766. Because no significant changes in locomotor and explorative behaviors (ambulation, rearing) were found with these peptides, it seems likely that the effect is specific for social behavior in isolated rats and not due to a general sedative effect. A similar effect has recently been described for anti-depressant drugs (5), indicating that these peptides mimic the action of these drugs in this test procedure. The site and mode of action of these peptides on social behavior is unclear. Intensifications of catecholaminergic and serotonergic processes by PLG and TRH have been reported (24,25,26). Isolation has been shown to induce metabolic changes, at least for serotonin, in some brain areas related to the limbic system (17,27), that have been implicated in the affective modulation of behavior in animals and man (6). Thus, PLG, TRH and ORG 2766 may induce changes in catecholaminergic and/or serotonergic activity within the limbic system and this may be the underlying mechanism of the effects of these peptides on the changes in social behavior due to the isolation procedure. However, the decrease in social activity in isolated rats by these peptides may also be due to an interaction with endogenous opioid systems. Recently we have found that this effect of ORG 2766 is naltrexone reversible (28) and evidence has been presented that PLG and ORG 2766 may directly or indirectly interact with opioid systems (29,30,31,32,33).

Moreover, the opioid peptide β-endorphin increases the social activity of isolated rats. The effect on social interactions by β-endorphin is however different from that observed with PLG, TRH and ORG 2766. β-Endorphin predominantly affects contact behavior, while the other peptides decreased all social activities in the isolated animals. This suggests that different systems may be involved in the peptide induced changes in social behavior. Although α-endorphin and DTγE (peptides generated from β-endorphin by brain enzymes (11), like β-endorphin increase social interactions of the isolated rats, the action of α-endorphin and DTγE does not resemble that of β-endorphin. In fact, α-endorphin increased all social interactions especially during the second half of the test period and DTγE increased particularly the frequency of social explorative behavior during the first part of the test session (data not shown). It has been reported that α-endorphin possesses in addition to its opiate like action psychostimulant (amphetamine like) properties which are located in the sequence 2-9 of the molecule (34, 35). Because neither this fragment (β-E 2-9) nor DTγE did affect social behavior in a similar way as found for β-endorphin, this psychostimulant-like quality of α-type endorphins may not be responsible for the effect of α-endorphin on social behavior.

In addition to its effect in isolated animals, β-endorphin also increased social interactions when injected in non-isolated animals. As in isolated animals, the effect was most pronounced on the item contact behavior. A decrease in amicable social behavior has been found by Meyerson (36) in male-male encounters after intracerebroventricular injection with 5 µg/animal β-endorphin but not with 1 µg/animal, while this latter dose increased this behavior in male rats in male-female encounters. Kavaliers (37) showed that intracerebroventricular treatment with low doses of β-endorphin (5 pg/g body weight) increased schooling behavior in goldfish, while a higher dose (15 pg/g) induced an opposite effect. The increased schooling behavior by β-endorphin was naloxone reversible. All these data suggest that β-endorphin very potently affects social behavior which may implicate this peptide in physiological processes underlying this behavior.

No significant effects of α-MSH, ACTH 1-24, ACTH 4-10 and (D-Phe^{7})-ACTH 4-10 were observed in the present experiments, but γ-MSH significantly decreased the total amount of social interactions in non-isolated animals. It has been reported that γ-MSH decreased territorial aggression in rats (38) and that ACTH suppressed isolation-induced aggression in albino mice (39,40). Recently, a decrease in active social contact following treatment with ACTH 1-24 and ACTH 4-10 has been found, when rats were tested under special conditions (41,42,12). Thus, in general ACTH-related peptides may decrease social interactions. Interestingly, the effects of ACTH 4-10 and γ-MSH in the present study are opposed to the effects obtained with β-endorphin particularly with respect to social contact behavior. Recently, it was concluded that γ-MSH, at least in some aspects, may operate as functional antagonist of β-endorphin (43), while a similar conclusion has been drawn for ACTH 1-24 and related peptides (44, 45,46,7). The present data imply that such a functional antagonism between β-endorphin and ACTH-related peptides, particularly γ-MSH, may also exist with respect to social behavior.

In conclusion, neuropeptides can influence social behavior of rats in doses that do not affect locomotor or explorative behavior. Certain neuropeptides like TRH,PLG and ORG 2766 decrease the enhanced social activity in short-term isolated animals and mimic in this respect the action of antidepressant drugs. However, more experimentation is needed before these peptides can be labelled as peptides with antidepressant activity. β-Endorphin, α-endorphin and DTγE increase social interactions in rats after peripheral administration. Especially of interest is that β-endorphin is very potent in this respect, which may be of significance for the implication of opioid brain systems in social behavior. Consistently, the effect of β-endorphin in social contact behavior is blocked by the opioid antagonist naltrexone (13). β-Endorphin affects social behavior of non-isolated rats in a way opposed to ACTH 4-10 and γ-MSH, suggesting that a possible functional antagonism between these peptides may play a role in social behavior.

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