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Short communication

Facilitation and attenuation of social recognition in rats by different oxytocin-related peptides

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Abstract

The memory-modulating effects of subcutaneously administered oxytocin and related peptides were investigated in the social recognition paradigm in rats. The attenuation of social recognition by 0.6 and 6 μg/kg of oxytocin was mimicked by the same doses of des-Gly^8-oxytocin, [pGlu^4,Cyt^6]oxytocin-(4–9) and prolyl-leucyl-glycinamide (PLG) and by 0.6 μg/kg of oxytocin-(1–6) and [pGlu^4,Cyt^6]oxytocin-(4–8). The peptide leucyl-glycinamide (LG) was not active in this respect. The facilitation of social recognition by 0.6 and 6 ng/kg of oxytocin was mimicked by the same doses of PLG and LG but not by the other peptides and the amino acid glycine. It is concluded that the active moieties for attenuation and facilitation of social memory reside in different parts of the oxytocin molecule, e.g. the 5–7 and 8–9 region, respectively.

Keywords: Social recognition; Social memory, rat; Oxytocin; PLG (prolyl-leucyl-glycinamide); Neurohypophyseal hormone; Structure activity

1. Introduction

The nonapeptide oxytocin affects many peripheral and central physiological functions. For example, pregnancy, labor and pup feeding are regulated by this peptide (Caldeyro-Barcia et al., 1971), and oxytocin is implicated in the regulation of feeding as well as sexual behavior (reviewed in Caldeyro-Barcia et al., 1971). Oxytocin has also been implicated in learning and memory processes. The peptide, when given in pharmacological doses to animals, attenuates memory and thus has been regarded as an amnestic peptide (Schulz et al., 1974). This effect has been found in an avoidance test (Van Ree et al., 1978) and in the social recognition test (Dantzer et al., 1987; Popik and Vetulani, 1991). In this latter procedure, the duration of social interactions of an adult male rat with a juvenile stimulus rat is measured and the reduction in duration of social investigation on subsequent encounters is used as an index of social memory. This memory is present when the inter-exposure interval lasts 30 min or less (Thor and Holloway, 1982). Recently using the social recognition test, we found that whereas high, 'pharmacological' doses (> 24 ng–6.0 μg/kg) of oxytocin attenuated social recognition (Popik and Vetulani, 1991), low, 'physiological' doses (0.09–6.0 ng/kg) of the peptide facilitated social recognition (Popik et al., 1992). Similar facilitating effects of oxytocin on social recognition were found when relatively low doses were injected into the medial preoptic area of the hypothalamus in rats (Popik and Van Ree, 1991). In addition, low doses of systemically administered oxytocin facilitated another form of social learning, i.e. social transmission of flavored fluid preference (Popik and Van Ree, 1993).

Since the memory-attenuating effects of oxytocin, as assessed with the avoidance test procedure, are mimicked by several oxytocin-derived peptides (Burbach et al., 1983; De Wied et al., 1987), while other analogues possess memory-facilitating effects (Walter et al., 1975), it was of interest to perform structure-activity studies with oxytocin-derived peptides in the ethologically relevant social recognition paradigm in which both memory facilitation and attenuation can be investigated (Thor and Holloway, 1982).

Two different test procedures were used. In one procedure high doses of the peptides were tested, using a 30-min inter-exposure interval, which allows assessment of the attenuation of social recognition. The other procedure was

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aimed to investigate facilitation of social recognition: low doses of the peptides were tested using a 120-min inter-exposure interval. In both procedures, two doses of each peptide were tested.

2. Material and methods

2.1. Animals and test procedure

The rats (residents) whose social recognition was studied ($n = 58$) were retired breeders, 6-month-old male retired breeders Wistar rats weighing 450–500 g. Juvenile intruders, 3- to 4-week-old male rats weighing 80–100 g, served as recognition subjects. The residents were housed alone and the juveniles in groups of 6 in the same experimental room, under a reversed light–dark cycle (light off 7 a.m.–7 p.m.), for a few weeks before and during the experiment. Tap water and commercial food were available ad libitum. The residents were used repeatedly, with an interval of at least 2 days between testing. The juveniles were replaced every 7th day to avoid aggressive behavior from the residents. The test consisted of placing a juvenile in the home cage of the resident, where the encounter was observed. The encounter lasted 300 s. At the end of the first encounter, the juvenile was removed and the resident was injected with peptide solution or placebo. After an inter-exposure interval of 30 or 120 min, the same juvenile was presented to the resident for the second encounter. During encounters, the total Social Investigation Time of the resident towards the juvenile was measured in seconds, using an IBM-PC compatible computer. Social investigation included social sniffing, social grooming, close following and crawl-over behaviors (Popik et al., 1992).

Residents were subjected to a pre-experimental training (a few exposures to different juveniles on consecutive days with varying inter-exposure intervals) (Dantzer et al., 1987). Only those rats that displayed no aggressive or sexual behavior towards juveniles, and for which a time-response relationship could be determined, were used in the experiments (Popik et al., 1992).

2.2. Drugs

Oxytocin [oxytocin-(1–9)], desglycinamide-oxytocin [oxytocin-(1–8)], tocinamide [oxytocin-(1–6)]-NH_2, TOC), Pro-Leu-Gly-NH_2 [oxytocin-(7–9], PLG), Leu-Gly-NH_2 [oxytocin-(8–9], LG), glycine, [$pGlu^4$,Cyt^6]oxytocin-(4–8) [oxytocin-(4–8)], and [$pGlu^4$,Cyt^6]oxytocin-(4–9)NH_2 [oxytocin-(4–9)] were diluted with physiological saline just before the experiment. The peptides were injected subcutaneously (neck) in a volume of 1 ml/kg. The doses used for assessing the recognition facilitating effects were 0.6 and 6.0 ng/kg (except glycine which was used in doses of 1.0 and 10 ng/kg). The recognition attenuating effects were assessed with doses of 0.6 and 6.0 μg/kg. The selection of these doses was based on previous studies (Popik et al., 1992; Popik and Vetulani, 1991). Each peptide treatment was placebo controlled, using a cross-over design. Half of the rats were injected with the peptide solution, while the other rats received placebo (saline). This treatment was reversed on the second test day with the same animals. In this way each rat received placebo and a peptide treatment. Each dose of a peptide was given to 8–11 residents. The experimenter was not aware of the treatment conditions. The peptides were kindly donated by Organon International, Oss, Netherlands.

2.3. Data presentation and statistics

The change in social interest is expressed as the Recognition Index, which was calculated as the social investigation time in the second encounter divided by the social investigation time in the first encounter and multiplied by 100. The data are presented as the placebo-controlled Recognition Index, which was calculated as the Recognition Index of peptide treatment subtracted from the Recognition Index of placebo treatment for each individual animal. Thus, positive values for placebo-controlled Recognition Index indicate facilitation, while negative values indicate attenuation of social recognition. The mean and S.E.M. of the Recognition Indexes and placebo-controlled Recognition Indexes were computed. To evaluate statistically effects of peptides on social recognition, the values of the Social Investigation Times (in seconds) for each peptide and respective placebo treatments were subjected to a two-way, within-subject analysis of variance. A significant interaction between the injection (placebo vs. peptide) and encounter (first vs. second) factors was considered a treatment effect.

3. Results

The rats engaged in vigorous social interaction during both encounters. The Social Investigation Time of the residents towards juveniles varied between 80 and 120 s, which is around 30% of the exposure time.

Table 1 presents the recognition facilitating and attenuating effects of oxytocin-related peptides. Treatment with high doses (0.6 and 6.0 μg/kg) of oxytocin-(1–9), oxytocin-(1–8), oxytocin-(7–9), and oxytocin-(4–9) decreased the social recognition. The peptides oxytocin-(1–6) and oxytocin-(4–8) showed a similar action, but the amnestic effects were less pronounced and present only at one dose (0.6 μg/kg). Oxytocin-(8–9) was not effective in this respect at either dose tested. When rats were tested after an inter-exposure interval of 120 min and treated with the low doses of the peptides (0.6 and 6.0 ng/kg), oxytocin-(1–9), oxytocin-(7–9) and oxytocin-(8–9) facilitated the social

Table 1
Attenuation (at inter-exposure interval (IEI) of 30 min) and facilitation (at IEI of 120 min) of social recognition by various oxytocin-related peptides

Treatment	Placebo-controlled Recognition Index			
	IEI 30 min		IEI 120 min	
	0.6 μg/kg	6 μg/kg	0.6 ng/kg	6 ng/kg
Oxytocin, [oxytocin-(1–9)]:	−29.6 ± 9.1 [a]	−88.9 ± 20.9 [c]	37.8 ± 13.6 [b]	43.4 ± 13.9 [b]
H–Cys–Tyr–Ile–Gln–Asn-Cys-Pro–Leu–Gly–NH_2				
Des-Gly[9]-oxytocin, DGoxytocin, oxytocin-(1–8)]:	−53.5 ± 20.4 [b]	−53.8 ± 16.9 [b]	9.6 ± 18.3	4.9 ± 13.3
H–Cys–Tyr–Ile–Gln–Asn–Cys-Pro–Leu–OH				
Tocinamide, [oxytocin-(1–6)]:	−22.0 ± 7.3 [a]	−27.7 ± 9.6	3.2 ± 5.9	0.6 ± 6.7
H–Cys–Tyr–Ile–Gln–Asn–Cys-NH_2				
Prolyl-leucyl-glycinamide, PLG, [oxytocin-(7–9)]:	−47.0 ± 15.1 [a]	−71.2 ± 25.7 [a]	55.9 ± 23.6 [a]	32.9 ± 10.9 [a]
H–Pro–Leu–Gly–NH_2				
Leucyl-glycinamide, LG, [oxytocin-(8–9)]:	11.8 ± 16.1	0.5 ± 10.2	19.7 ± 8.4 [a]	27.6 ± 10.7 [a]
H–Leu–Gly–NH_2				
Glycine	Not tested	Not tested	6.7 ± 26.4	−16.1 ± 24.3
[pGlu[4],Cyt[6]]oxytocin-(4–9), [oxytocin-(4–9)]:	−43.3 ± 8.1 [c]	−21.6 ± 5.9 [a]	1.2 ± 9.8	9.4 ± 8.5
H-Cys-OH pGlu–Asn–Cys–Pro–Leu–Gly–NH_2				
[pGlu[4],Cyt[6]]oxytocin-(4–8), [oxytocin-(4–8)]:	−24.2 ± 10.2 [a]	−14.8 ± 21.0	5.6 ± 10.0	−8.7 ± 8.1
H-Cys-OH pGlu–Asn–Cys–Pro–Leu-OH				

The drugs were given just after the first encounter. Presented are means ± S.E.M. of the placebo-controlled Recognition Index (see Material and methods). a, b or c indicates a significant attenuation or facilitation, computed as a significant interaction factor in the two-way, within-subject ANOVA for each drug dose: [a] $P < 0.05$; [b] $P < 0.01$, [c] $P < 0.001$. Glycine was given at the doses of 1.0 and 10 ng/kg.

recognition; the other tested peptides and glycine were ineffective in this respect.

4. Discussion

The present results indicate that the attenuation and facilitation of social recognition by oxytocin reside in different parts of the molecule. The attenuation induced by high doses of oxytocin was mimicked by oxytocin-related peptides with and without the C-terminal glycinamide. The attenuation of social recognition by oxytocin-(1–9), oxytocin-(1–8), oxytocin-(1–6), oxytocin-(7–9), oxytocin-(4–9), and oxytocin-(4–8), but not by oxytocin-(8–9), indicates that the region 5–7 of the oxytocin molecule is particularly important for this action. The facilitation induced by low doses of oxytocin was mimicked only by peptides with the C-terminal glycinamide, i.e. oxytocin-(1–9), oxytocin-(7–9) and oxytocin-(8–9). Thus, the active moiety for the facilitating action may reside in the 8–9 region of oxytocin, although it is puzzling why oxytocin-(4–9) was not active in this respect. Control experiments have shown that the high doses of oxytocin do not facilitate social recognition (Popik et al., 1992). In the present series of experiments, we tested also a high dose of oxytocin-(7–9) in the procedure with an inter-exposure interval of 120 min: social recognition was not facilitated (Recognition Index and placebo-controlled Recognition In-

dex were 113.7 ± 20.7 and -17.0 ± 27.2, respectively, $n = 7$). Thus, the facilitation of social recognition is restricted to low doses (nanograms per kg) of the peptides.

Memory-attenuating effects of oxytocin-related peptides have been observed in avoidance test procedures. Structure-activity studies have revealed that the peptides [pGlu^4,Cyt^6]-oxytocin-(4–8) and [pGlu^4,Cyt^6]-oxytocin-(4–9) are active in this respect (De Wied et al., 1987). This corroborates the present findings that these peptides attenuated social recognition. Of interest is that such peptides can probably be generated from oxytocin by enzymatic processes (Burbach et al., 1983).

Small C-terminal peptides of oxytocin have also been implicated in memory processes. Most studies have used the C-terminal tripeptide prolyl-leucyl-glycinamide (PLG, oxytocin-(7–9)), which is also termed MSH (melanocyte-stimulating hormone)-release inhibiting factor (MIF). Walter et al. (1975) found that several C-terminal oxytocin-derived peptides, including PLG and leucyl-glycinamide (LG, oxytocin-(8–9)), attenuated puromycin-induced amnesia in mice. PLG facilitated learning of a reversal of brightness discrimination in albino rats (Rigter and Popping, 1976). In other studies PLG facilitated the development of physical dependence on morphine, an effect which was interpreted in the context of learning and memory (Van Ree and De Wied, 1976; Van Ree et al., 1978). Memory-attenuating effects of PLG and related peptides have also been found. For example, the peptide facilitated extinction of conditioned taste aversion (Rigter and Popping, 1976). Also other types of effects of PLG have been described, e.g. on social behavior (Niesink and Van Ree, 1984), opiate antagonistic effects (Kastin et al., 1979) and modulation of reward (Van Ree and De Wied, 1977; Dorsa and Van Ree, 1979). However, it should be stressed that all the above mentioned effects were observed with doses much higher than those used in the present study. Regarding the effectiveness of low doses of PLG and LG in facilitating social recognition, it may be postulated that these or structurally related peptides are physiologically involved in memory processes. Moreover, the present studies provide evidence that the attenuating and facilitating actions of oxytocin-related peptides on social memory are mediated by different mechanisms and/or receptor systems.

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