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NEUROPEPTIDES RELATED TO NEUROHYPOPHYSEAL HORMONES INTERFERE WITH APOMORPHINE-INDUCED BEHAVIORAL CHANGES

Xin-sheng Xiao, H. Dick Veldhuis and Jan M. Van Ree

Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands
(Reprint requests to JMVR)

ABSTRACT

The interaction between peptides related to neurohypophyseal hormones and brain dopaminergic systems was studied by investigating in rats the effect of these peptides on behavioral changes induced by graded doses of the specific dopamine agonist apomorphine. Low doses of this drug induce hypoactivity of the animals, while higher doses result in hyperactivity and stereotyped sniffing. Desglycinamide^{9}[Arg^{8}]vasopressin (DG-AVP), prolyl-leucyl-glycinamide (PLG) and oxytocin did not interfere with the behavioral responses induced by the higher doses of apomorphine. Peptide treatment made the rats more sensitive to apomorphine with respect to the drug induced hypoactivity. PLG and especially DG-AVP were more effective than oxytocin. It is concluded that these peptides may have a selective action on distinct dopaminergic receptor systems in the brain, that are presumably located presynaptically in the nucleus accumbens area.

INTRODUCTION

Evidence is accumulating that neuropeptides related to the neurohypophyseal hormones vasopressin and oxytocin affect brain processes (1-3). Thus, these neuropeptides influence memory processes, attenuate experimentally induced amnesia, affect the development of tolerance to morphine, change acquisition of heroin self-administration and modify the electrical activity of the brain. It has been postulated that these effects are mediated by an interaction of these neuropeptides with brain catecholamine systems (2,4). Indeed, systemic, intracerebroventricular and intracerebral administration of these peptides affect dopaminergic and noradrenergic activities in various brain areas (5-9). These peptides may be involved in the physiological control of catecholamine neurons, because temporary bioinactivation of vasopressin by injecting vasopressin antiserum into the cerebrospinal fluid changes catecholamine activities in several brain areas (10). The mechanism by which these peptides exert this physiological control remains however to be elucidated.

We have approached this question by investigating the interaction of neuropeptides related to neurohypophyseal hormones with the behavioral ef-

fects of the specific dopamine agonist apomorphine. This drug induces hypoactivity after low doses and hyperactivity and stereotypy after high doses (11). One explanation of this biphasic effect is that low doses preferentially activate presynaptically located dopaminergic receptor systems, leading to diminished dopamine release, while high doses activate postsynaptically located dopaminergic receptor systems. As peptides we selected desglycinamide^{9}[Arg^{8}]vasopressin (DG-AVP), which induces similar behavioral effects as vasopressin, but lacks the classical peripheral actions of the hormone (12), oxytocin which exerts an opposite effect as vasopressin in a variety of behavioral testprocedures (2) and prolyl-leucyl-glycinamide (PLG), the C-terminal tripeptide of oxytocin. In particular PLG has been shown to interfere with brain dopaminergic system (8,13,14). We found that especially DG-AVP and PLG potentiated the behavioral effects of low doses of apomorphine, but did not interact with the behavioral responses following high doses of this drug, indicating that these peptides interfere with certain but not all dopaminergic systems in the brain and suggesting that they may act at the presynaptic level of dopaminergic neurons.

METHODS

Animals and test conditions

Male Wistar rats weighing 140-160 g were used. They were housed under controlled conditions and maintained on a 14:10 light-dark cycle (light on between 5:00 a.m. and 7:00 p.m.) with free access to food and water. Experiments were carried out between 8:30 a.m. and 2:00 p.m. in a sound-attenuated room. Neuropeptides and apomorphine were administered subcutaneously in the neck of the animals. Each animal was used only once.

A rectangular (A) and a circular (B) perspex test cage were used to observe the behavior of the animals as described in detail elsewhere (11). Briefly, groups of animals (n=6-42) were injected with saline (0.5 ml) or neuropeptides and after 1 h with placebo (0.3 ml saline) or graded doses of apomorphine. Five min later, the rats were placed in (A) the rectangular test cage (bottom 20 x 6.5 cm, height 32 cm) and the frequency of locomotion, rearing and grooming was measured for 3 min. The rats were tested again in (B) the small open field (diameter 19.5 cm, height 28.5 cm) at 20 min after the last injection and the frequency of locomotion and rearing and the duration of sniffing were measured for 3 min.

Data analysis and statistics

Group means and standard errors were calculated and the statistical significance was determined using one-way analysis of variance (ANOVA) and if significant ($P<0.01$), post hoc comparisons of group means were evaluated with the Student Newman-Keuls multiple range test (SNK, SPSS program).

Drug and peptides

Apomorphine (Apomorphine HCl) was obtained from O.P.G. Utrecht, The Netherlands. The neuropeptides desglycinamide^{9}[Arg^{8}]vasopressin (DG-AVP, Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg), oxytocin (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2) and PLG (Pro-Leu-Gly-NH_2) were donated by Organon International B.V., Oss, The Netherlands. The peptides were stored in dry form and under controlled humidity condition. Peptides and apomorphine were dissolved in saline immediately prior to use.

RESULTS

Low doses of subcutaneously injected apomorphine decreased the frequency of locomotor activity and rearing both at 5 and 20 min after injection (Fig. 1). The relatively high dose (250 μg/kg) of apormophine increased the frequency of locomotion and rearing but only at 20 min after administration. Low doses of apomorphine did change neither the frequency of grooming nor the duration of sniffing. Higher doses however, decreased grooming and induced stereotyped sniffing behavior.

The influence of pretreatment (25 μg, s.c.) with the neuropeptides (DG-AVP, oxytocin and PLG) is presented in Table 1. Neither peptides affect the measured behavioral elements in placebo treated rats. Injection of a low dose of apomorphine (31.25 μg/kg) induced a significant decrease of locomotor activity in DG-AVP and PLG pretreated rats as compared to saline pretreated controls. This effect was less marked and statistically nonsignificant in oxytocin pretreated rats. Also the frequency of rearing was significantly decreased in DG-AVP pretreated rats, while a tendency to a similar effect was found in the PLG pretreated rats. Thus, DG-AVP and to a minor degree PLG clearly potentiate the effects of low dose of apomorphine on locomotion and rearing. In fact, DG-AVP shifted the dose response curve of apomorphine to the left (Fig. 2). Using a lower dose of DG-AVP, i.e. 2.5 μg, a slight, but nonsignificant effect was observed (data not shown). Grooming behavior was affected neither by apomorphine treatment nor by the combined treatment of neuropeptides and apomorphine. However, the duration of sniffing behavior was lower in all rats pretreated with all three peptides as compared to saline pretreated controls following the low dose of apomorphine.

When higher doses of apomorphine were injected no differences were observed between saline and peptides pretreated rats (Table 1). Of particular interest is that the increase in locomotion and rearing and the occurence of stereotyped sniffing behavior elicited by high doses of apomorphine were not significantly affected by any of the neuropeptides.

DISCUSSION

The present data confirm previous studies showing that low doses of apomorphine decrease locomotor activity and rearing, while higher doses increase locomotor activity and rearing and induce stereotyped sniffing (11). That the increase of the behavioral elements measured is not present at 5 min after apomorphine treatment is probably due to a lower level of apomorphine in the brain as compared to 20 min after drug administration (15). The different and even opposing behavioral changes induced by apomorphine are likely related to activation of different dopaminergic systems in the brain. It has been shown that low doses of apomorphine injected into the nucleus accumbens decrease locomotion and rearing (16,17), while injection of higher doses in the same area increases locomotion and rearing (18,19). Injections of neither low nor high doses of apomorphine into the nucleus caudatus change locomotion and rearing activities (18). In contrast, high doses of apomorphine injected into the nucleus caudatus, but not in the nucleus accumbens, elicit stereotyped sniffing, probably by activation of postsynaptically located receptor systems (18,20). Thus, dopaminergic systems present in the nucleus accumbens and nucleus caudatus may mediate the apomorphine-induced changes in locomotor activity and rearing and the drug-induced stereotypy respectively (21,22). Concerning locomotor activity and rearing, low doses of apomorphine decrease while higher doses increase these behavioral elements. It has been argued before that these differential effects may be mediated by stimulation of presynaptically and postsynaptically located dopaminergic receptor systems respectively (11,17).

TABLE 1.

The influence of pretreatment with different neuropeptides on apomorphine-induced behavioral changes

Treatments		number of	Rectangular (A)			Circular (B)		
-60 min	0	rats	locomotion	rearing	grooming	locomotion	rearing	sniffing (sec.)
saline	placebo	42	9.8 ± 0.5+	8.7 ± 0.5	7.4 ± 0.9	16.4 ± 1.0	8.4 ± 0.6	38 ± 2
DG-AVP		36	8.5 ± 0.5	7.4 ± 0.4	8.0 ± 1.2	16.1 ± 0.7	8.4 ± 0.5	41 ± 2
oxytocin		30	9.5 ± 0.5	8.2 ± 0.6	7.9 ± 1.1	16.3 ± 1.1	9.1 ± 0.6	44 ± 2
PLG		36	8.5 ± 0.4	8.0 ± 0.5	5.5 ± 0.8	14.9 ± 0.6	9.1 ± 0.6	42 ± 2
saline	apomorphine	18	9.2 ± 0.6	6.4 ± 0.5	7.1 ± 1.4	14.4 ± 1.0	6.7 ± 0.7	40 ± 2
DG-AVP	(31.25	12	5.2 ± 0.6*	3.5 ± 0.4*	6.4 ± 1.6	9.1 ± 1.0*	2.9 ± 0.6*	32 ± 4*
oxytocin	µg/kg)	6	7.3 ± 0.7	6.0 ± 0.5	6.2 ± 3.3	11.7 ± 1.0	7.2 ± 1.4	29 ± 2*
PLG		12	6.4 ± 0.6*	4.8 ± 0.7	6.8 ± 1.5	9.8 ± 0.9*	6.3 ± 0.7	28 ± 2*
saline	apomorphine	18	7.5 ± 0.6	4.8 ± 0.6	7.1 ± 1.5	10.8 ± 0.8	5.4 ± 0.6	38 ± 4
DG-AVP	(62.5	12	5.2 ± 0.7	3.5 ± 0.5	5.8 ± 1.8	13.2 ± 1.3	4.9 ± 0.6	37 ± 3
oxytocin	µg/kg)	6	7.8 ± 1.0	6.5 ± 0.4	7.3 ± 1.9	13.3 ± 1.0	6.0 ± 0.9	33 ± 1
PLG		12	7.7 ± 0.9	5.1 ± 0.6	8.3 ± 1.9	16.3 ± 1.8	6.4 ± 1.0	36 ± 3
saline	apomorphine	12	5.8 ± 0.6	4.5 ± 0.6	2.6 ± 0.8	15.9 ± 1.9	7.4 ± 1.1	51 ± 5
DG-AVP	(125 µg/kg)	6	4.7 ± 0.9	1.7 ± 0.3	5.3 ± 1.6	11.7 ± 0.9	5.2 ± 0.8	47 ± 4
PLG		6	6.7 ± 1.6	4.0 ± 1.4	6.3 ± 2.3	19.5 ± 4.2	7.5 ± 1.1	48 ± 5
saline	apomorphine	18	7.1 ± 0.5	5.3 ± 0.4	3.8 ± 1.1	34.0 ± 2.3	15.0 ± 1.0	88 ± 3
DG-AVP	(250 µg/kg)	18	6.3 ± 1.0	3.9 ± 0.7	2.6 ± 0.7	28.6 ± 2.2	13.8 ± 1.5	75 ± 3
oxytocin		18	6.2 ± 0.7	3.9 ± 0.6	4.3 ± 1.2	27.2 ± 2.7	12.8 ± 1.2	77 ± 4
PLG		18	5.1 ± 0.6	3.7 ± 0.6	3.3 ± 1.0	27.4 ± 2.9	11.7 ± 1.5	82 ± 5

Rats were subcutaneously treated with saline (0.5 ml) or 25 µg DG-AVP, oxytocin or PLG and after 1 h with placebo or graded doses of apomorphine. They were tested in rectangular (A) and circular (B) testbox for 3 min at 5 and 20 min after the last injection respectively.

+ Mean ± SEM

* $P<0.05$ vs saline-apomorphine (31.25 µg/kg) treated rats (Student Newman-Keuls multiple range test).

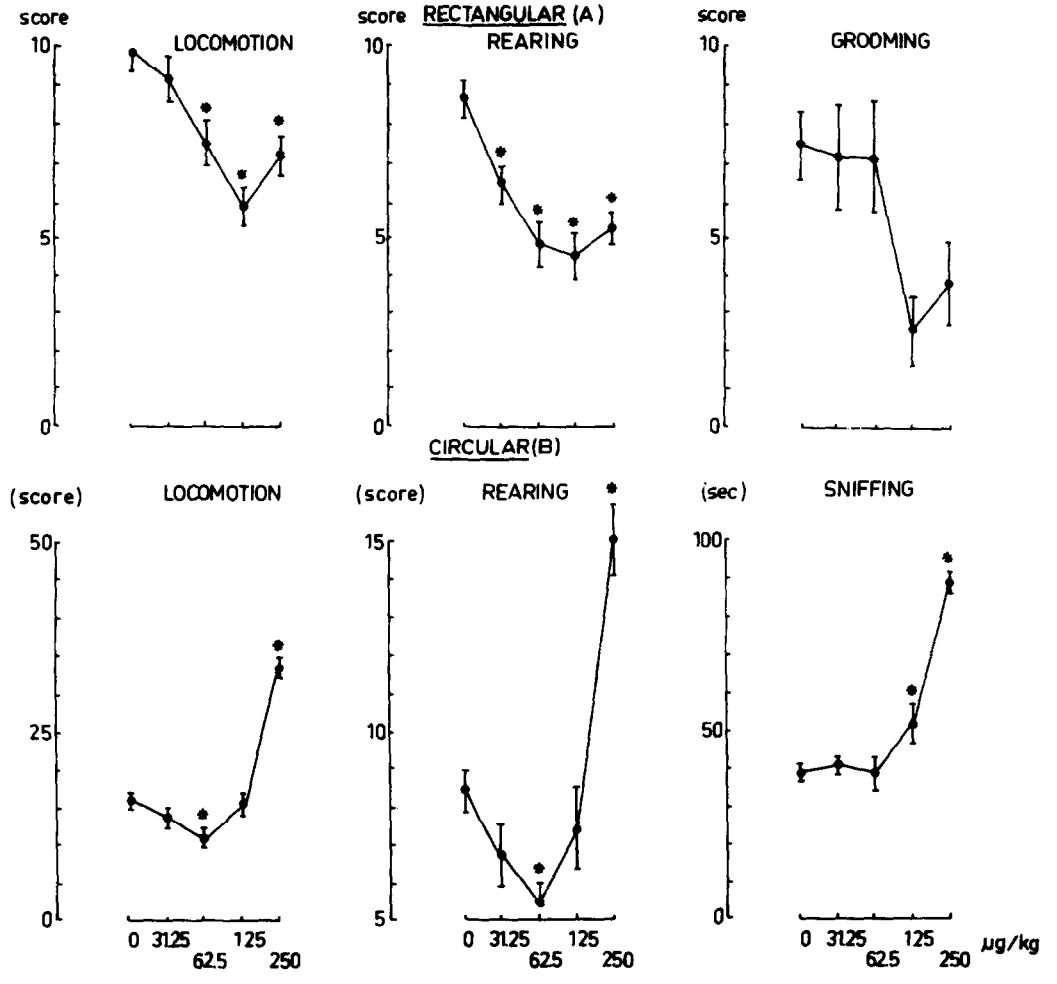

Fig. 1. Apomorphine-induced behavioral changes as assessed in a rectangular (A) cage for 3 min starting 5 min after subcutaneous treatment with placebo or graded doses of apomorphine and tested in a circular (B) cage for 3 min starting 20 min after treatment. Animals were injected subcutaneously with saline 1 h before apomorphine.
Mean behavioral scores versus the dose of apomorphine is presented. Vertical bars represented 2 SEM.

*$P<0.05$ vs placebo treated rats (Student Newman-Keuls multiple range test).

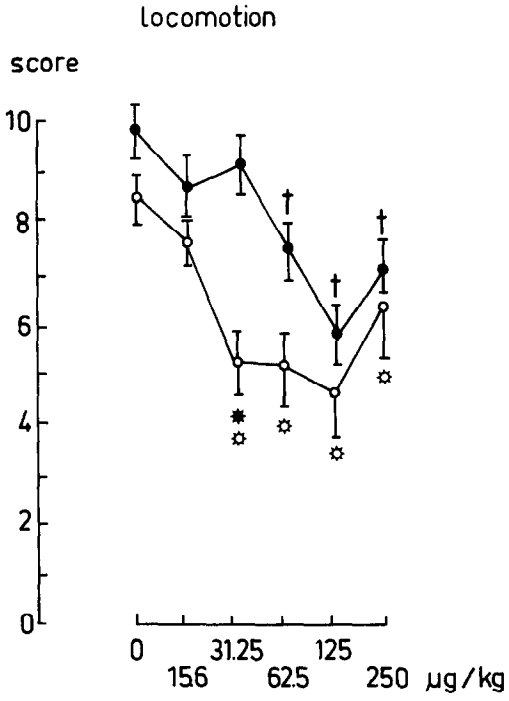

Fig.2. Effect of subcutaneous pretreatment with saline (0.5 ml) (●——●) or 25 µg DG-AVP(o——o) on apomorphine-induced decrease of locomotion. Groups of animals were subcutaneously injected with placebo or graded doses of apomorphine 1 h after pretreatment and 5 min before testing in a rectangular test cage. The mean locomotion score versus the dose of apomorphine is presented. Vertical bars represent 2 SEM.

* P<0.05 vs saline-apomorphine (31.25 µg/kg) treated rats (Student Newman-Keuls multiple range test).

☼† P<0.05 vs saline-placebo, respectively DG-AVP-placebo treated rats (Student Newman-Keuls multiple range test).

None of the tested peptides attenuated or potentiated the increase of locomotion and rearing and the stereotyped sniffing response induced by relatively high doses of apomorphine. This suggests that the peptides do not interfere with postsynaptic receptor systems probably located in the nucleus accumbens and nucleus caudatus and activated by apomorphine leading to the mentioned changes in behavior. The peptides did however interfere with the behavioral effects induced by low dose of apomorphine. Both DG-AVP and PLG significantly potentiated the apomorphine induced decrease in locomotion and rearing. A tendency towards a similar effect was found for oxytocin. DG-AVP appeared the most effective in this respect. Considering the dose response curves of apomorphine in the absence and presence of the peptide, it can be concluded that the rats pretreated with peptides are more sensitive to apomorphine with respect to drug induced hypoactivity. Thus, the tested peptides, especially DG-AVP and PLG may interfere with dopaminergic receptor systems presumably presynaptically located in the nucleus accumbens. Whether the decrease of sniffing behavior observed in rats treated with peptides and a low dose of apomorphine is also due to an interaction of the peptides with presynaptically located dopaminergic receptor systems, probably elsewhere in the brain, is not clear as yet.

A number of neurochemical studies has been performed dealing with the interaction between neurohypophyseal hormones and their fragments with catecholaminergic activity in the brain. The reported data are however difficult to interprete since the effects seem to depend on the dose of the peptide, the time that elapsed after peptide administration, the brain structure investigated, the method of determination of catecholaminergic activity, etc. (4). Thus, whether neurochemical data can support the interaction between neurohypophyseal peptides and presynaptically located dopaminergic systems, as suggested by the present findings, can not be answered at the moment. Other behavioral studies dealing with the tested peptides and apomorphine, are limited to PLG. It has been shown that PLG can potentiate the apomorphine-induced stereotypy and turning behavior (23,24,25). However, these effects were observed in rats in which the dopaminergic receptor systems were supersensitive due to either selective denervation by 6-OHDA or chronic treatment with haloperidol. Consistent with our data, in naive animals PLG failed to influence the effect of high doses of apomorphine on climbing activity in mice (25) and apomorphine-induced stereotypy in rats (26). However, in general much higher doses of PLG were used in the mentioned studies as compared to the present experiments. Cox (27) showed that PLG (1 mg/kg) slightly but significantly antagonized apomorphine-induced stereotypy in rats, which is not inconsistent with the suggestion of a potentiation of presynaptic dopaminergic receptor activation. That neurohypophyseal hormones and their fragments may interfere with brain dopamine at the presynaptic level, has also been suggested by Schulz (28) who studied the action of these peptides on rotation behavior following unilateral 6-OHDA-induced lesion of the dopaminergic cell bodies in the substantia nigra.

It has been shown that vasopressin, oxytocin and PLG have different and even opposing effects in certain behavioral testprocedures (e.g. extinction of active avoidance behavior, passive avoidance behavior, acquisition of heroin self-administration, electrical self-stimulation behavior from the ventral tegmental area) (2,29). However, these peptides induce the same effects in other testprocedures, for example, these peptides facilitate the development of tolerance to and physical dependence on morphine and attenuate experimentally induced amnesia (2). It remains however to be shown that the potentiating effect of the peptides on apomorphine-induced hypolocomotion is somehow related to their influence on amnesia and morphine action.

In conclusion, the present data show that peptides related to neurohypophyseal hormones i.e. DG-AVP, oxytocin and PLG interfere with brain dopaminergic receptor systems. This action is,however,restricted to certain dopaminergic systems. No interaction was found with apomorphine-induced hyperlocomotion and stereotyped sniffing, responses probably mediated by postsynaptically located dopaminergic receptor systems in the nucleus accumbens and caudatus respectively. Peptide treated rats were more sensitive to apomorphine with respect to the drug-induced hypoactivity, suggesting that the peptides interfere with specific dopaminergic receptor systems, presumably located presynaptically in the nucleus accumbens area. In this respect, PLG and especially DG-AVP were more effective than oxytocin.

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