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Facilitatory Effects of Monoamine Synthesis Inhibitors on Lysine-Vasopressin Induced Changes in the Exploratory Behaviour Pattern of Male Rats

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HÖGLUND, A. U. AND B. J. MEYERSON. *Facilitatory effects of monoamine synthesis inhibitors on lysine-vasopressin induced changes in the exploratory behaviour pattern of male rats*. PHARMACOL BIOCHEM BEHAV **21**(6) 859–863, 1984.— The possible interaction between vasopressin and monoamines in the regulation of exploratory behaviour was investigated. Treatment with lysine-vasopressin (LVP) influenced this behaviour. In particular, the investigative activity was enhanced. The dose-response relationship of LVP was non-linear. The catecholamine synthesis inhibitor α-methyl-p-tyrosine (α-MT, 100 mg/kg) also increased the investigative activity, indicating a suppressive influence of catecholamines on this behaviour. α-MT pretreatment potentiated the effect of LVP within certain dose levels. The dose-response relationship for the peptide effect on the investigative activity was shifted towards the left. Treatment with the serotonin biosynthesis inhibitor para-chloro-phenylalanine (PCPA) (100+50+50 mg/kg) also enhanced the investigative behaviour and potentiated the action of LVP similarly to α-MT. It is suggested that the action of LVP on the exploratory behaviour in rats can be modified by monoamines.

Monoamines Indoleamines α-Methyl-p-tyrosine p-Chlorophenylalanine Lysine-vasopressin Exploratory behaviour Habituation

PARVOCELLULAR neurons containing vasopressin (VP) project to different areas of the telencephalon and diencephalon of the brain from the suprachiasmatic nucleus [7, 11, 13, 24]. These projections of VP-containing neurons imply that VP can, by a direct action, modify limbic activities.

Many attempts have been made to evaluate the possibility of a vasopressinergic action on behaviour. It is mostly the hypothesis that this peptide influences learning and memory processes that has been tested [2, 3, 4, 16, 21, 28, 29]. It has been shown that, despite its rapid degradation [10], subcutaneously administered VP retards the extinction of both a passive and an active avoidance response [3,4]. It is suggested from these studies that vasopressin influences long-term memory [29]. Similar results have also been obtained after injections of small amounts of the peptide into the cerebral ventricles [4] or local application of small amounts in different brain areas [16]. In contrast, however, some authors have reported that VP has no influence on consolidation processes [5, 14, 22]. Carey and Miller found no deficit in learning and memory in an active or passive avoidance task in Brattleboro rats [5] after treatment with this peptide. Hostetter *et al.* found that subcutaneously (SC) administered lysine-vasopressin (LVP) had no effect on passive avoidance behaviour [14]. Sahgal *et al.* observed that intracerebroventricularly (ICV) injected arginine-vasopressin exerted a bimodal effect and suggested that VP acts on motivational rather than consolidation processes [22].

In addition to influencing measures associated with learning and memory processes VP has been found to affect the pattern of non-operant behaviour in rats such as grooming [6] and exploratory behaviour [12]. One component of the exploratory behaviour is investigative activity. This activity increases both in duration and in frequency during habituation to an unfamiliar situation. This increase is facilitated by VP [12].

Although numerous reports have been made concerning the influence of VP on behaviour, the mechanism by which the peptide acts in this respect is so far unknown. Indications have been found that LVP exerts a modulating effect on catecholaminergic neurons [18,19]. The dopamine (DA) turnover increases in the striatum and septum, while that of noradrenaline increases in the hypothalamus, when the peptide is administered SC [18]. ICV injections of arginine-vasopressin increase the nerve impulse activity in noradrenergic neurons in the hypothalamus, thalamus and medulla oblongata [26].

Alpha-methyl-p-tyrosine (α-MT) has been found to pre-

vent the facilitatory action of VP on passive avoidance behaviour [18], suggesting that the cerebral catecholaminergic system might be one of the major mediators of the behavioural action of VP. Para-chlorophenylalanine (PCPA) impaired passive avoidance behaviour [17], indicating the possibility that serotonin may also be implicated.

The aim of the present study was to further investigate the possible interaction between LVP and monoaminergic neurotransmission with regard to the influence on the exploratory behaviour in the rat, especially the LVP-induced effects on the investigative activity.

The dose-response relationship of the LVP effects on exploratory behaviour was determined, as a basis for evaluation of the influence of impaired monoaminergic transmission. Similarities were found in the effects of LVP and the influence of the monoamine synthesis inhibitors α-MT [27] and PCPA [15]. α-MT and PCPA was found to influence the effect of LVP on exploratory behaviour. A co-function between LVP and monoaminergic mechanisms is discussed.

METHOD

Animals and Laboratory Conditions

Male Sprague-Dawley rats, weighing 250–300 g were used. They were housed in steel cages (30×20×50 cm) with five animals in each cage, and were provided with commercial rat pellets (Ewos R3) and tap water ad lib. The temperature was kept at 21±1°C. The day-night cycle was reversed, with light between 21.00 hr and 09.00 hr. All tests were performed between 13.00 hr and 17.00 hr under conditions of dimmed light (less than 5 lux).

Exploratory Behaviour Test

The test period was 10 min, during which time the animal moved about freely, exploring its environment (a plywood box with a Plexiglas front and a floor covered with wood shavings). The duration (the total time for which a behaviour was performed), frequency (the number of times a behaviour was performed) and latency (the time from the start of recording to the first occurrence of a behaviour) of three different elements of the exploratory behaviour were recorded by means of a computer. The behaviours recorded have been described in detail elsewhere [20] but the main points will be recapitulated briefly here.

Sniffing. Rapid movements of whiskers while the animal explores. Scores were taken regardless of whether the animals were sniffing while moving, or sniffing while stationary.

Rearing. Standing on hind legs while sniffing.

Investigating. Intense sniffing directed at a particular object, which is picked up by the animal and explored.

Experimental Schedule

Three groups of animals were tested. All animals were allowed to explore the observation box for 10 min each day for three days (days 1, 2, 3) before the pre-experimental trial (day 4). One hour before the experimental trial (day 7) the first group of animals received different SC doses of LVP (Ferring, 250 IU vasopressor activity/mg, 0, 0.05, 0.5, 2.0, 5.0 μg/kg SC). The second group received α-MT (Labkemi, 100 mg/kg IP) 5 hr before the experimental trial. One hour before this trial the animals were given LVP as in the first group. The third group was treated with PCPA (Labkemi) for three days (100+50+50 mg/kg IP) with the last injection 5 hr before the experiment trial. One hour before this trial these animals also received LVP as in the other groups. The time schedule is further outlined in Fig. 1.

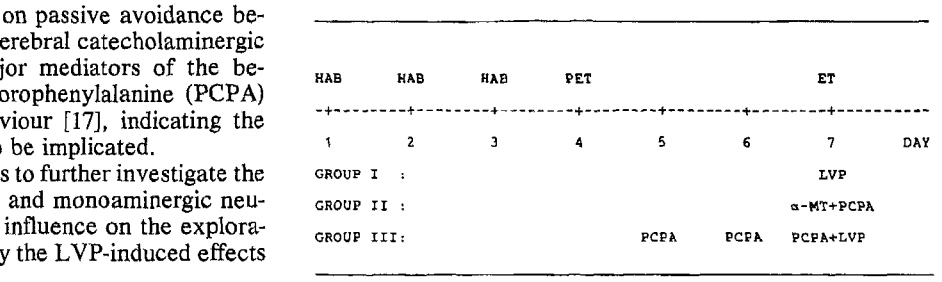

FIG. 1. Experimental schedule. Abbreviations: HAB=habituating trial, PET=pre-experimental trial, ET=experimental trial, LVP=lysine-vasopressin, PCPA=p-chlorophenylalanine, α-MT=α-methyl-p-tyrosine.

Statistical Treatment

The significances of the differences between groups were calculated by the Mann-Whitney U test, with a level of significance, α, of 0.05 with the two-tailed region of rejection [23]. The Kruskal-Wallis one way analysis of variance was used to test differences within groups.

RESULTS

The administration of LVP resulted in a change in exploratory behaviour (Table 1). The main effect of this treatment was exerted on the investigative activity, which increased in duration and frequency and decreased in latency. The dose-effect relationship of LVP was not linear. There was some increase in response from 0.05 to 0.5 μg/kg and further increase between 2.0 and 5.0 μg/kg. The LVP induced change in the investigative activity seemed to occur in two phases along the dose axis.

The treatment with a α-MT (Table 2) alone significantly increased the duration and frequency of investigative activity, and decreased the frequency of rearing and the duration of sniffing. The pretreatment with α-MT altered the dose-response relationship of LVP so that the doses 0.05 and 2.0 μg/kg significantly increased the duration of investigating, while doses of 0.5 and 5.0 μg/kg did not cause any significant change in this respect. The frequency of investigating increased at 2.0 μg/kg. Together with the increase in the investigative performance, there were also changes in the other behaviours recorded. The frequency of sniffing and rearing decreased significantly after an LVP dose of 0.5 μg/kg.

Treatment with PCPA (Table 3) alone resulted in a change in the exploratory behaviour similar to that produced by α-MT. Thus, the duration and frequency of investigating increased significantly. The duration and frequency of rearing decreased and the latency of this behaviour increased. The pretreatment with PCPA changed the dose-response relationship of LVP in the same manner as did the α-MT treatment. Like α-MT, the pretreatment with PCPA also brought about a decrease in sniffing and rearing at certain LVP levels. The duration of rearing decreased at LVP doses of 0.5 and 5.0 μg/kg and its frequency decreased after 0.5 μg/kg. Simultaneous with the dramatic increase in the duration of

TABLE 1

GROUP I: THE EFFECT OF VARIOUS DOSES OF LYSINE-VASOPRESSIN (LVP) ON THE EXPLORATORY BEHAVIOUR OF THE RAT

LVP Dose	Sniffing			Rearing			Investigating			
μg/kg	DUR.	FRE.	LAT.	DUR.	FRE.	LAT.	DUR.	FRE.	LAT.	
0.00	−5.2	1.9	0.0	2.6	0.1	−6.2	6.5	1.7	−82.0	15
0.05	−11.5	1.5	−0.1	3.6	0.1	−2.7	11.6	3.2	−81.9	15
0.50	−15.8	5.3	0.0	0.2	1.1	1.4	22.4	6.2	−123.4	15
2.00	−21.9	−4.9	0.0	2.1	−9.0	−3.8	23.7	5.2	−79.3	15
5.00	−21.3	−1.4	0.0	−24.2	−6.6	0.7	46.9	10.3	−170.4	15
H	3.2	8.0	6.5	9.1	14.4††	5.4	32.0†††	30.3†††	6.8	
PET	441.9	53.0	0.6	111.8	48.0	5.4	15.7	6.0	190.7	15

Differences between experimental and pre-experimental values. PET represents pre-experimental trial values in the control group. Kruskal-Wallis analysis of variance (H) was used to test differences between groups (†$p<0.05$, ††$p<0.02$, †††$p<0.001$). N denotes the number of animals in each group. Durations (DUR.) and latencies (LAT.) are given in seconds and frequencies (FRE.) as scores.

TABLE 2

GROUP II: THE EFFECT OF LYSINE-VASOPRESSIN (LVP) ON THE EXPLORATORY BEHAVIOUR OF THE RATS AFTER PRETREATMENT WITH α-METHYL-P-TYROSINE (100 mg/kg)

LVP Dose	Sniffing			Rearing			Investigating			
μg/kg	DUR.	FRE.	LAT.	DUR.	FRE.	LAT.	DUR.	FRE.	LAT.	
0.00	−27.3*	−3.4	0.0	−2.0	−6.4*	1.1*	21.6*	4.7*	−59.1	15
0.05	−38.4	1.3	0.0	8.7	−2.2	1.2	40.2**	6.7	−63.0	15
0.50	−19.5	−4.8**	0.0	−7.2	−9.0**	−3.4	24.2	5.0	−104.4	15
2.00	−48.8*	−4.4	0.1	−14.2	−13.0	−1.6	53.8**	11.3**	−149.8	15
5.00	−28.8*	−9.5	0.0	−20.1	−16.5	4.8	53.9	8.6	−117.5	15
H	4.2	4.5	2.7	7.6	7.3	6.1	11.3†	8.3	5.0	
PET	459.3	61.0	0.8	82.8	53.0	2.6	27.1	9.0	169.9	15

The Mann-Whitney U-test was used to test differences between corresponding doses of LVP in Groups I (Table 1) and II, where *$p<0.05$, **$p<0.02$, ***$p<0.001$. See also legend to Table 1.

TABLE 3

GROUP III: THE EFFECT OF LYSINE-VASOPRESSIN (LVP) ON THE EXPLORATORY BEHAVIOUR OF THE RATS AFTER PRETREATMENT WITH PARA-CHLORO-PHENYLALANINE (100 + 50 + 50 mg/kg GIVEN ON THREE SUCCESSIVE DAYS)

LVP Dose	Sniffing			Rearing			Investigating			
μg/kg	DUR.	FRE.	LAT.	DUR.	FRE.	LAT.	DUR.	FRE.	LAT.	
0.00	−26.3	−6.8	0.0	−17.3*	−10.0*	8.8**	20.9**	3.7*	−29.8	15
0.05	−21.3	1.1	0.0	−6.2	−5.2	−5.9	26.6**	5.2	−93.6	15
0.50	−38.4	−10.3**	0.0	−28.4**	−15.1*	−0.9	17.6	2.8*	−28.9*	15
2.00	−63.4**	−1.9	0.0	−7.3	−7.4	−3.2	55.4**	9.6	130.2	15
5.00	−49.1	−8.2	0.0	−54.6*	−20.0	4.9	36.6	10.5	−49.7*	15
H	6.4	4.6	1.7	11.2†	8.5	10.1	7.7	7.3	5.1	
PET	454.4	58.0	0.2	91.9	47.0	2.6	25.3	10.0	170.3	15

The results were compared with those after corresponding doses of LVP in Group I (Table 1). For statistical treatment and explanations of symbols, see legends to Tables 1 and 2.

investigative activity the duration of sniffing decreased after treatment with PCPA combined with 2.0 mg/kg of LVP. The frequency of sniffing decreased after LVP, dose of 0.5 μg/kg.

DISCUSSION

Usually the term habituation is used when a response to a specific stimulus decreases. In the exploratory test situation it may be assumed that the anxiety about the unfamiliar situation will diminish when the animal is repeatedly introduced to the observation box. This decrease in anxiety will result in expression of behaviour which initially have had long latencies.

In an earlier study we found that the investigative activity increased during the habituation to the observation box [12]. We also showed that LVP was capable of increasing both the duration and frequency of investigative behaviour while its latency decreased. These two findings together led to the hypothesis that LVP might facilitate habituation.

In the present study some further features of LVP when used in an exploratory behaviour situation were demonstrated. Thus, LVP was found to have a non-linear dose-response relationship, with an increase at 0.5 and 5.0 μg/kg. The data of Tanaka *et al.* [26] indicate a bimodal dose-response relationship with respect to the effects of arginine-vasopressin on the DA content in different brain areas after ICV injection of the peptide. Another example of a biphasic effect of peptides was reported by Gaffori *et al.* [8], who found that the γ-type endorphins exhibited an inverted U-shaped dose-response curve regarding passive avoidance behaviour.

Pretreatment with α-MT influenced the dose-response relationship of LVP. This effect of α-MT was, however, dependent upon the dose of LVP. Thus, α-MT did not add any effect to that of LVP when the peptide was given in doses of 0.5 and 5.0 μg/kg. With LVP doses of 0.05 and 2.0 μg/kg, however, an additive effect was observed. One explanation for these findings may be that the α-MT treatment shifts the LVP dose-response relationship towards the left.

Since a decreased catecholaminergic synthesis caused an increased investigative activity it is reasonable to assume that this behaviour is under inhibitory influence of catecholamines. This leads to a further possibility that LVP interacts with catecholamines concerning this inhibition. Thus, LVP may act by eliminating or diminishing this inhibitory effect. The non-linear dose-response relationship suggests, however, that more than one mechanism may be involved; for example, different monoaminergic transmitter systems with perhaps different functional effects, such as inhibitory and facilitatory ones respectively. Another possibility to be considered is that LVP and the catecholaminergic mechanisms are two separate systems, acting independently. The decreased catecholamine biosynthesis would thereby lead to a potentiating effect on the stimulatory action of LVP.

The pretreatment with PCPA also shifted the dose-response curve of LVP towards the left. The interpretation of these results is complicated by the fact that PCPA, in addition to its effects on serotonergic biosynthesis, also influences the action of catecholamines. Disappearance of NA when PCPA is given in doses exceeding 300 mg/kg has been reported by Alexander [1]. Lower doses do not, however, lead to irreversible inactivation of tryptophan hydroxylase, as Gal reported [9]. Koe and Weissman [15] and Tagliamonte [25] used repeated injections of PCPA (3×100 mg/kg) which led to a decrease in 5-HT levels without any significant reduction of NA. The possibility cannot be ruled out at present that the quantitative change in response to LVP induced by pretreatment with PCPA may be an "α-MT like" effect. As a rather low dose was used in our experiments, it seems possible, on the other hand, that only 5-HT is decreased and that this transmitter is responsible for the observed effect. It is nevertheless possible that both catecholamines and serotonin are involved in the changes in the exploratory behaviour occurring after administration of LVP.

Our findings are contradictory to earlier reports [17,18] where a decreased monoaminergic synthesis prevented the action of LVP. One explanation to this discrepancy may be that there are differences between the behavioural responses measured. Another plausible explanation is that shift in the dose-response relationship has been responsible for the prevented effect of LVP.

In summary, the results of this study demonstrate the ability of LVP to influence exploratory behaviour in the rat and in particular the investigative activity. Furthermore, the data reveal that the monoamine synthesis inhibitors α-MT and PCPA are able to influence the effect of SC administered LVP on exploratory behaviour. A co-function between LVP and monoaminergic neurotransmission is suggested, although the direct mechanism of the interaction and the specific monoamines involved are still unclear.

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