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Microinjection of anti-vasopressin serum into limbic structures of the rat brain: effects on passive avoidance responding and on local catecholamine utilization

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Rats which had received bilateral microinjections of 1:50 diluted anti-vasopressin serum into the dorsal or ventral hippocampus, immediately after the learning trial of a one-trial passive avoidance test, showed a reduction in avoidance latency scores during subsequent retention tests 24 and 48 h later. Postlearning microinjection of anti-vasopressin serum into either the dorsolateral septum or the caudate nucleus was without effect on the retention of passive avoidance behavior. Microinjection of anti-vasopressin serum 1 h before the 24-h retention session into either the dorsal hippocampus, the ventral hippocampus or the dorsolateral septum attenuated avoidance responding during both the 24-h and 48-h retention sessions, whereas preretention microinjection of the serum into the caudate nucleus was not effective. Intracerebroventricular administration of the anti-vasopressin serum in amounts similar to those used in the microinjection experiments did not affect retention scores when given either immediately after the learning trial or before the first retention session. One week after the behavioral experiments, a repeated microinjection of anti-vasopressin serum decreased the local α-methyl-*p*-tyrosine methylester (α-MPT)-induced disappearance of noradrenaline in the ventral hippocampus and the dorsal hippocampus respectively. Microinjection of the antiserum in the dorsolateral septum enhanced noradrenaline disappearance in this brain region. No effect was found on α-MPT-induced dopamine disappearance in the caudate nucleus following local microinjection of anti-vasopressin serum. These data show that endogenous vasopressin in both the dorsal and the ventral hippocampus is functionally involved in consolidation processes as well as in retrieval processes related to passive avoidance behavior, while that in the dorsolateral septum seems to be involved in retrieval processes only. They also show that noradrenergic mechanisms in these 3 brain regions respond to the local reduction of the amount of bioavailable vasopressin in a direction opposite to that observed after local microinjection of vasopressin, which suggests that vasopressin might act by modulating noradrenergic neurotransmission.

INTRODUCTION

A variety of central nervous system effects of vasopressin have been reported (for reviews see refs. 9,11,23,26,33,35). The effects of vasopressin on the retention of passive avoidance behavior and the possible involvement of endogenous vasopressin in rat brain in this process have been extensively studied since the first report by Ader and De Wied[1]. Facilitation of passive avoidance behavior has been observed when the peptide was administered systemically or intracerebroventricularly (i.c.v.) either immediately after the learning trial (postlearning treatment) or shortly before the retention session (preretention treatment)[1,4]. Based on the finding that postlearning microinjection of vasopressin into the dorsal hippocampus, dorsal septum and dorsal raphe nucleus[15], and postlearning as well as preretention microinjection of the peptide into the ventral hippocampus[19], resulted in longer avoidance latencies during subsequent retention sessions, it was suggested that limbic-midbrain structures are the anatomical substrate for the effects of vasopressin on passive avoidance behavior[13–16,19,29].

One approach to study the physiological involvement of endogenous neuropeptides in brain function is to study the effects of i.c.v. administration or local microinjection of antisera to these neuropeptides in

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order to reduce the amount of bioavailable peptide in the brain (for reviews see refs. 29,32). Using this approach it was found that i.c.v. administration of anti-vasopressin serum had effects on the retention of passive avoidance behavior opposite to those of treatment with vasopressin[30,31]. Postlearning local microinjection of anti-vasopressin serum into either the dorsal raphe nucleus[18] or the dorsal hippocampus[17] attenuated passive avoidance responding 24 h later. Again, the direction of the effect was opposite to that observed after local microinjection of vasopressin, suggesting a role of endogenous vasopressin in these brain regions in passive avoidance behavior[15,17–19].

The experiments described in this communication were carried out to extend the above-mentioned observations and to obtain information on the effects on avoidance responding of microinjections of anti-vasopressin serum into various limbic-midbrain regions, using postlearning as well as preretention treatment schedules. In addition, the effects of microinjections of anti-vasopressin serum were studied on local catecholamine utilization, to further investigate possible correlates between behavioral influences and neurochemical effects of locally reduced levels of vasopressin[13,15].

A preliminary report of some of these data has been presented during the Winter Neuropeptide Conference (January 1985, Breckenridge, CO)[34].

MATERIALS AND METHODS

Animals

Male Wistar albino rats, weighing 160–180 g, were kept under a standard illumination schedule of 14 h light–10 h dark (lights on at 06.00 h). Standard laboratory food and tap water were available ad libitum. The rats were housed 5 to a cage. The behavioral experiments were performed in a sound-attenuated room between 08.00 and 13.00 h.

Surgery

All surgical procedures were performed under fluanison-fentanyl (Hypnorm, 0.06 ml per 100 g body weight) anesthesia.

For i.c.v. injections, rats were equipped with a polyethylene cannula in the lateral cerebral ventricle as described by De Wied[8]. After insertion into the ventricle, the cannula was fixed to the skull with dental cement. The behavioral studies were carried out at least 6 days after surgery. The localization of the tip of the cannula was determined at the termination of the experiments by macroscopical inspection of the brain ventricular system after i.c.v. injection of Evans blue.

For local microinjections, rats were equipped with intracerebral cannulas as described previously[15]. Stainless steel guide cannulas (external diameter 0.66 mm) were implanted bilaterally, according to the coordinates of König and Klippel[12], into one of the following brain structures: the dorsal dentate gyrus of the hippocampus (P 3.7, L 3.5 and D 3.8; under an angle of 20° from the vertical (90°) position); the ventral hippocampus (P 4.3, L 5.1 and D 7.7; under an angle of 40°); the dorsolateral septum (A 1.0, L 1.8 and D 4.7; under an angle of 12°); and the caudate nucleus (A 2.2, L 2.8 and D 5.0; under an angle of 5°). A recovery period of 7 days was allowed before the behavioral experiments were performed. Injections were given to freely moving rats through an internal cannula which was inserted via the implanted guide cannula. Localization of the tips of the cannulas was determined microscopically on 300 μm thick frozen brain sections. Data obtained with rats with an improper cannula placement were discarded.

Passive avoidance behavior

Passive avoidance behavior was studied in a one-trial learning, step-through type, passive avoidance test situation[2], which utilizes the natural preference of rats for a dark environment. The apparatus consisted of a dark compartment with a grid floor and an illuminated, elevated platform attached to its front center. On day 1, the rats were habituated to the dark compartment for 2 min. Immediately after habituation, the rats were placed on the platform and allowed to enter the dark compartment. Three more such trials were given on the following day. At the end of the third trial, an inescapable electric footshock (0.5 mA, AC, for 2 s) was delivered through the grid floor of the dark compartment as soon as the rats entered the dark compartment (learning trial). The rats were removed from the dark compartment 10 s after the termination of the shock. Retention of passive avoidance was tested 24 and 48 h after the learning trial by measuring the latency to enter the dark compartment. Latencies were measured to a maximum of 300 s.

Treatment

Anti-vasopressin serum with a binding capacity of approximately 2.5 ng [Arg^8]vasopressin per μl, raised in rabbits as previously described[17,31], was used after 1:50 (v/v) dilution with normal rabbit serum. Two μl of this 1:50 dilution was injected into the lateral ventricles, while 1 μl was injected bilaterally into the cannulated brain regions (i.e. 1 μl per side). As control serum normal rabbit serum was used. Injections were given immediately after the learning trial (postlearning treatment) or 1 h prior to the 24 h retention session (preretention treatment) in the behavioral experiments.

Local catecholamine utilization

Seven days after the behavioral experiment, α-methyl-*p*-tyrosine methylester-HCl (α-MPT, AB Biotec, Göteborg; 250 mg/kg) was given i.p. at $t = 0$ min, followed, 30 min later, by the local microinjection of either 1 μl of the 1:50 dilution of anti-vasopressin serum or of 1 μl of normal rabbit serum (controls) into the brain region into which this same treatment had been given in the behavioral study. Three hours after the local microinjection, the rats were killed by decapitation and the brain was rapidly excised and immediately frozen on dry ice. The brains were cut in 300-μm sections at a temperature of −10 °C. The brain regions into which the sera were injected were dissected from the frozen sections according to Palkovits[22]. The tissue pellets were then homogenized in 130 μl ice-cold 0.1 M $HClO_4$, containing 3,4-dihydroxybenzylamine as the internal standard, followed by centrifugation at 10,000 *g* for 20 min at 4 °C. The supernatants were transferred to minivials containing 10 μl 0.5 M sodium acetate to raise the pH to 3.5. The samples were stored at −80 °C until used for analysis. The samples were analyzed using a Hewlett Packard series 1090 chromatograph equipped with a Metrohm electrochemical detector, as described elsewhere[36]. The results are expressed as pg catecholamine per μg protein ± S.E.M. The protein content was measured using a micro-modification of the method of Lowry et al.[21].

Statistics

The behavioral data were analyzed using Mann–Whitney's non-parametric ranking test (two-tailed). The neurochemical data were analyzed by a two-tailed Student's *t*-test. A probability level of 5% or less was accepted as a significant difference.

TABLE I

Effect of 1:50 (v/v) diluted anti-vasopressin (anti-AVP) serum on the retention of passive avoidance behavior following i.c.v. administration and local microinjection immediately after the learning trial

Injection volumes of the 1:50 (v/v) diluted anti-vasopressin serum and the control serum were: 2 μl i.c.v., and 1 μl bilaterally locally into the indicated brain structures. [a] Median latencies are given with, in parentheses, the 25–75% percentiles.

Treatment	*n*	*Avoidance latency (s)*	
		During the 24-h retention session	*During the 48-h retention session*
I.c.v.			
control serum	6	300 (262–300)[a]	300 (214–300)
anti-AVP serum	6	290 (275–300)	274 (198–300)
Local			
Dorsal hippocampus			
control serum	6	175 (125–300)	160 (145–175)
anti-AVP serum	7	10 (8–11)*	15 (12–18)*
Ventral hippocampus			
control serum	8	247 (136–300)	285 (157–300)
anti-AVP serum	8	24 (43–230)*	75 (34–246)*
Dorsolateral septum			
control serum	13	220 (40–300)	214 (45–300)
anti-AVP serum	12	245 (143–300)	117 (48–300)
Caudate nucleus			
control serum	6	250 (215–300)	248 (221–300)
anti-AVP serum	8	232 (81–300)	189 (70–300)

* $P < 0.05$ for difference with control serum-treated group (Mann–Whitney's non-parametric ranking test, two-tailed).

RESULTS

Rats which had been treated i.c.v. with a 1:50 dilution of the anti-vasopressin serum in a volume of 2 μl, either immediately after the learning trial (Table I) or shortly before the 24-h retention session (Table II), showed avoidance latency scores at the 24-h and 48-h retention sessions which were not different from those of rats treated with control serum.

When 1 μl of the 1:50 diluted anti-vasopressin serum was bilaterally microinjected locally into either the dorsal hippocampus or into the ventral hippocampus, rats showed a pronounced reduction in avoidance latencies during the 24-h and 48-h retention sessions. This was observed after postlearning treatment (Table I) as well as after preretention treatment (Table II). Local microinjection of anti-vasopressin serum into the dorsolateral septum immediately after the learning trial was without effect on avoidance latencies during later retention sessions (Table I). Preretention microinjection of the antiserum into this brain region, however, did cause a reduction of the avoidance latency scores at both the 24-h and 48-h retention sessions (Table II). Microinjection into the caudate nucleus did not affect avoidance latencies after either postlearning or preretention treatment (Tables I and II).

Table III shows the data on the effects of local microinjections of anti-vasopressin serum on local noradrenaline or dopamine utilization as compared to that after local treatment with control serum. A decreased utilization of noradrenaline was evident in the dorsal hippocampus as well as in the ventral hippocampus following local microinjection of anti-vasopressin serum, as can be seen from the significantly higher noradrenaline concentration in these brain regions of anti-vasopressin-treated rats 3.5 h after α-MPT administration (Table III). An opposite effect, i.e. an increase in noradrenaline utilization after treatment with anti-vasopressin serum, was found in the dorsolateral septum (Table III). In all 3 brain regions, the dopamine concentration 3.5 h after α-MPT administration was below the detection limit of the method. After microinjection of anti-vasopressin serum into the caudate nucleus, no difference was found in local dopamine utilization in this region as compared to that of rats treated with control serum (Table III). No noradrenaline was detected in the caudate nucleus 3.5 h after α-MPT.

TABLE II

Effect of 1:50 (v/v) diluted anti-vasopressin (anti-AVP) serum on the retention of passive avoidance behavior following i.c.v. administration and local microinjection one hour prior to the 24-h retention session

Injection volumes of the 1:50 (v/v) diluted anti-vasopressin serum and the control serum were: 2 μl i.c.v., and 1 μl bilaterally locally into the indicated brain structures. [a] Median latencies are given with, in parentheses, the 25–75% percentiles.

Treatment	*n*	*Avoidance latency (s)*	
		During the 24-h retention session	*During the 48-h retention session*
I.c.v.			
control serum	11	300 (300–300)[a]	300 (300–300)
anti-AVP serum	14	300 (300–300)	300 (240–300)
Local			
Dorsal hippocampus			
control serum	9	260 (90–280)	220 (193–276)
anti-AVP serum	8	25 (18–32)*	42 (31–53)*
Ventral hippocampus			
control serum	11	218 (155–300)	134 (117–196)
anti-AVP serum	19	28 (9–119)*	29 (17–98)*
Dorsolateral septum			
control serum	7	218 (155–300)	187 (134–196)
anti-AVP serum	9	16 (12–62)*	29 (14–98)*
Caudate nucleus			
control serum	7	209 (111–243)	218 (142–227)
anti-AVP serum	7	236 (87–300)	239 (64–300)

* $P < 0.05$ for difference with control serum-treated group (Mann–Whitney's non-parametric ranking test, two-tailed).

TABLE III

Effect of local microinjection of 1:50 (v/v) diluted anti-vasopressin (anti-AVP) serum on local α-MPT-induced catecholamine disappearance

For treatment schedule, see Materials and Methods. [a] Mean ± S.E.M.

Treatment	*n*	*Catecholamine*	*Catecholamine content 3.5 h after α-MPT administration (pg/μg protein)*
Dorsal hippocampus		Noradrenaline	
control serum	18		2.68 ± 0.31[a]
anti-AVP serum	23		3.69 ± 0.35*
Ventral hippocampus		Noradrenaline	
control serum	24		3.37 ± 0.17
anti-AVP serum	21		4.09 ± 0.25*
Dorsolateral septum		Noradrenaline	
control serum	20		3.01 ± 0.21
anti-AVP serum	22		2.01 ± 0.20*
Caudate nucleus		Dopamine	
control serum	14		12.83 ± 0.72
anti-AVP serum	14		12.55 ± 1.00

* $P < 0.05$ for difference with control serum-treated group (Student's *t*-test, two-tailed).

DISCUSSION

Based on the results of lesion studies and microinjection experiments, it has been postulated that limbic-midbrain structures, such as the hippocampus, septum and amygdala, are the anatomical substrate for the effects of vasopressin on avoidance behavior (for reviews see refs. 14,16,29,33). Furthermore, it has been put forward that catecholamine systems projecting to these structures are involved in the expression of the behavioral effects of vasopressin[13,14,16]. The present results are further support for these postulates. They show that endogenous vasopressin in both the dorsal hippocampus and the ventral hippocampus is functionally involved in consolidation processes as well as in retrieval processes, while that in the dorsolateral septum seems to be involved in retrieval processes only. They also show that noradrenergic mechanisms in these 3 brain regions respond to the local reduction of the amount of bioavailable vasopressin in a direction opposite to that after local elevation of the amount of vasopressin[15].

The observation that rats which had received a microinjection of anti-vasopressin serum into the dorsal hippocampus immediately after the training trial of a one-trial passive avoidance test showed a reduction in avoidance scores during subsequent retention tests, confirms the previous report by Kovács et al.[17]. These authors observed that, after microinjection into the dorsal hippocampus, the antiserum spread to the ventral part of the hippocampus and to the dorsolateral septum as well[17]. A high density of vasopressin binding sites has been found in all these 3 brain regions[3,7], with a localization corresponding to the localization of responsive sites in a behavioral and biochemical studies[15,17,19]. In our experiments we found postlearning administration of anti-vasopressin serum into the ventral hippocampus to be effective in attenuating the retention of passive avoidance responding, whereas postlearning microinjection into the dorsolateral septum was not. This, together with the finding that microinjection of low doses of [Arg[8]]vasopressin in either the dorsal[15,19] or the ventral[19] hippocampus has effects opposite to those which are observed after anti-vasopressin administration in these brain regions, suggests that endogenous vasopressin in both these parts of the hippocampus is involved in the consolidation of passive avoidance behavior. It is not known why a reduction in the amount of bioavailable vasopressin by postlearning injection of anti-vasopressin serum into the dorsolateral septum was without effect, whereas postlearning microinjection of [Arg[8]]vasopressin into this brain region did facilitate avoidance responding, as was shown by Kovács et al.[15]. A possible explanation might be that spreading of the injected substances to other brain regions has to be taken into account and that relatively low-molecular weight peptides, like vasopressin, are more likely to spread to effective

sites in the hippocampus than does anti-vasopressin serum.

The amount of the anti-vasopressin serum used in the microinjection experiments was without effect when administered i.c.v. immediately after the training session. This is in agreement with previous observations[17,30], strengthening the issue of the physiological significance of vasopressin present, and released in restricted brain areas.

Preretention treatment schedules have been used to study the effects of vasopressin and anti-vasopressin serum on avoidance latencies during the retention sessions, i.e. to study the possible involvement of vasopressin in the retrieval of stored information[28–30,33]. Microinjection of 1:50 diluted anti-vasopressin serum into either the dorsal hippocampus, the ventral hippocampus or the dorsolateral septum, in an amount which, following i.c.v. administration, was without effect, reduced avoidance latency scores during both the 24-h and the 48-h retention sessions. In all 3 cases the effect was similar to that observed after preretention i.c.v. administration of much larger amounts of anti-vasopressin serum[30], and opposite to that after the preretention i.c.v. administration of vasopressin[1]. As to data concerning local microinjections of vasopressin in a preretention treatment schedule, the only data are those of Kovács et al.[19], who reported a clear-cut facilitation of avoidance responding after vasopressin microinjection into the ventral hippocampus. This effect, again, is opposite to that observed in the present study following anti-vasopressin administration into this brain region. Thus, decreasing the amount of bioavailable vasopressin in dorsal hippocampus, ventral hippocampus or dorsolateral septum attenuates retrieval processes related to passive avoidance responding.

The fact that neither postlearning, nor preretention microinjection of anti-vasopressin serum into the caudate nucleus resulted in any change in later avoidance responding indicates that this brain region is not involved in either consolidation or retrieval processes. The caudate nucleus is only diffusely innervated by fibers of the extrahypothalamic vasopressin systems[5,20]. Vasopressin binding sites have not been detected in an appreciable density in the caudate nucleus[3,7]. An interaction of vasopressin with the nigrostriatal dopamine system on the level of the dopaminergic terminals in the caudate nucleus has been suggested on the basis of the results of behavioral and neurochemical studies[24,25,27]. No effect was found in the present experiments following microinjection of anti-vasopressin serum into the caudate nucleus on local utilization. This further supports that vasopressin's effects on caudate nucleus dopaminergic activity are indirect rather than direct[27].

Based on the finding that following destruction of the ascending noradrenergic bundle by bilateral microinjections of 6-hydroxydopamine, administration of [Arg^8]vasopressin is no longer effective in facilitating avoidance responding, it was postulated that the coeruleo-telencephalic noradrenaline system mediates the effects of vasopressin on passive avoidance learning[13–15]. Data in support of the notion that vasopressin might act by modulating noradrenergic neurotransmission in terminals of this system originate from microinjection studies and lesion experiments[13,15,18]. The present data are in line with this notion: the effects of local microinjections of anti-vasopressin serum into the dorsal hippocampus and the dorsolateral septum on local noradrenaline utilization are opposite to those previously observed after local microinjection of [Arg^8]vasopressin into these regions[13]. Noradrenaline utilization in the ventral hippocampus appears to be decreased to the same extent as that in the dorsal hippocampus. The effects of vasopressin, either endogenous to these brain regions or administered by microinjection, are not necessarily direct. In fact, neuronanatomical[5,6] as well as neurochemical[10] data argue against a direct interaction on the level of noradrenergic terminals. Neurochemical experiments are in progess to further study the mechanism of action with respect to vasopressin's effects on hippocampal and septal neurotransmission.

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