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FACILITATION OF MEMORY CONSOLIDATION BY VASOPRESSIN: MEDIATION BY TERMINALS OF THE DORSAL NORADRENERGIC BUNDLE?

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SUMMARY

Administration of arginine-vasopressin (AVP, 5 μg, s.c.) immediately after the learning trial results in a long-term facilitation of a one-trial learning passive avoidance response. This effect of AVP is absent in animals with prior destruction of the ascending dorsal noradrenergic bundle by bilateral microinjection of 6-hydroxydopamine (6-OHDA). Postlearning local microinjection of a minute amount of AVP via chronically implanted cannulae into the locus coeruleus did not influence passive avoidance behavior. Upon injection into the midbrain dorsal raphe nucleus, however AVP facilitated passive avoidance behavior. This effect, however, was absent in rats receiving previous microinjection of 5,6-dihydroxytryptamine (5,6-DHT) or of 6-OHDA into the dorsal raphe nucleus. Bilateral 6-OHDA-induced lesions of the nucleus accumbens or 5,6-DHT-induced destruction of the dorsal raphe nucleus did not prevent the effect of AVP administered subcutaneously.

The data suggest that vasopressin facilitates memory consolidation processes by modulating noradrenergic neurotransmission in terminals of the dorsal noradrenergic bundle. The serotoninergic neuronal network originating from the dorsal raphe nucleus has a secondary — norepinephrine-mediated — influence upon these processes.

INTRODUCTION

Vasopressin, a nonapeptide of hypothalamo-posterior pituitary origin facilitates memory consolidation and retrieval processes in laboratory animals and in men[14,23].

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[30]. Local application of minute amounts of arginine[8]-vasopressin (AVP) into certain limbic-midbrain structures also facilitates memory consolidation[22], supporting the suggestion based on lesion experiments[42], that limbic-midbrain structures are involved in the mediation of this effect.

Vasopressin treatment also affects cerebral norepinephrine metabolism[21,22,36,37]. Regional studies utilizing microdissection techniques[22,37] revealed a distinct pattern of alterations in cerebral catecholamine disappearance, changes being primarily localized in limbic-midbrain areas such as the dorsal septum, dentate gyrus, parafascicular nucleus, dorsal raphe nucleus and the locus coeruleus. In the majority of these structures, vasopressin accelerates norepinephrine disappearance.

Cerebral catecholaminergic (mainly noradrenergic) mechanisms have been implicated in learning and memory processes[10,18,28]. The question arose therefore, whether alterations of brain neurotransmitter metabolism elicited by vasopressin may be important for the action of the neuropeptide on memory consolidation processes. Most of the effects of vasopressin on catecholamine metabolism occur in brain structures which receive noradrenergic innervation from the A_6 catecholaminergic cell group (locus coeruleus) via the ascending dorsal noradrenergic bundle[12,16,19,38]. Therefore, our primary interest was focused on the dorsal noradrenergic bundle. We have investigated the effect of AVP on memory consolidation processes following 6-OHDA-induced destruction of this pathway. Since vasopressin has been reported to affect dopaminergic[21,22,36,37] and serotoninergic[33] neurotransmission as well and the dorsal noradrenergic bundle is known to interact with dopaminergic and serotoninergic systems[4,6,20,24,32,35], the role of the nucleus accumbens and of the dorsal raphe nucleus upon AVP-induced facilitation of memory consolidation has also been studied.

This paper presents evidence that the effect of vasopressin on memory consolidation processes is most probably mediated by the terminals of the dorsal noradrenergic bundle.

METHODS

Animals

Male rats of an inbred Wistar strain (CPB-TNO, Zeist, The Netherlands), weighing 160–180 g, were used. The animals were kept under standard illumination (light on between 5.00 and 19.00 h) with food and drinking water available ad libitum. The animals were housed 5 per cage.

Surgical methods

Animals were anaesthetized by fluanizon/fentanyl (Hypnorm, 0.06 ml/100 g) and were placed in stereotaxic instrument of 0° horizontal position, as described by Albe-Fessard et al.[2].

Acute microinjection of neurotoxic compounds. The skull was opened and a metal guide cannula was inserted in one of the following brain areas: dorsal noradrenergic bundle (P: 6.0; L: 0.6; V: 6.2; antero-posterior coordinate refers to bregma level,

vertical coordinate to the skull), nucleus accumbens (A: 3.0; L: 1.0; V: 6.7) or in the dorsal raphe nucleus (P: 7.2; L: 0.0; V: 6.6). Substances were injected via an internal cannula, protruding 0.1 mm from the guide cannula. 6-Hydroxydopamine (6-OHDA-hydrobromide, Sigma) or 5,6-dihydroxytryptamine (5,6-DHT-creatinine sulphate, Regis) were injected bilaterally (except for the dorsal raphe nucleus) in an amount of 10–10 μg, dissolved in 1.0 μl saline, containing 1% ascorbic acid. Sham-operated animals received the same volume of vehicle. The volume was injected over a period of 2 min. A recovery period of 10 days was allowed before starting the behavioral experiment.

Implantation of chronic cannula. Cannulae were implanted, as described in detail[22], in the dorsal raphe nucleus (coordinates see above) or bilaterally in the locus coeruleus (P: 10.2; L: 1.2; V: 7.2). Briefly, a guide cannula was inserted and cemented by acrylate to the bone. Neurotoxic compounds were injected via an internal cannula during the operation (for details see previous paragraph). Behavioral studies were started 10 days after the operation. Arginine[8]-vasopressin was injected via the same cannula in conscious, free-moving animals.

Localization of acute and chronic microinjections was controlled in histological sections upon completion of the experiments.

Behavioral methods

Passive avoidance behavior. Passive avoidance behavior was studied in a one-trial learning, step-through passive avoidance situation[1]. The apparatus consists of an illuminated platform, attached to a large dark compartment. The animals were placed on the platform and were allowed to enter the dark compartment (rats prefer dark to light). Three more trials were given on the following day. Unescapable electric footshock (0.25 mA AC for 2 sec) was delivered through the grid floor of the dark compartment. Retention of passive avoidance behavior was tested 24 and 48 h after the single learning trial by measuring the latency to re-enter the dark compartment up to a maximum of 300 sec.

Open field behavior. Open field behavior was measured 7 days after completion of passive avoidance experiments. As described in detail[45], ambulation (the number of floor units crossed), rearing, grooming and the number of fecal boli were measured in a circular arena for 3 min.

Neurochemical methods

Determination of catecholamine content. The animals were killed and the brains were rapidly taken out and frozen on dry-ice. Brains were cut in 300 μm sections at a temperature of —10 °C. Brain nuclei were removed as described earlier[22,31,37]. Tissue pellets were homogenized in 50 μl of 0.1 N $HClO_4$. A 10 μl aliquot was taken for protein assay[25]. The residual homogenate was centrifuged and norepinephrine and dopamine were determined from 20 μl aliquots of the supernatatant, using a radioenzymatic assay[40].

Determination of [^{3}H]5-HT uptake. Animals were killed and the brains were rapidly taken out. The mesencephalon and the dorsal hippocampi were dissected and

cut into slices with a tissue chopper. Tissue slices were preincubated (10 min, 37 °C) in Krebs–Henseleit buffer at pH 7.2. After preincubation, [^{3}H]serotonin (Amersham, England, spec. act. 37.4 Ci/mmol) was added to the incubation media, leading to a final concentration of 6.7×10^{-9} mol and the slices were incubated for 45 min at 37 °C. After centrifugation and washing, tissues were solubilized and the radioactivity was counted. In order to calculate non-specific uptake, another group of tissues was incubated at 0 °C in the presence of the same amount of radioactive serotonin.

Peptide treatment

Arginine^8-vasopressin (pressor activity 471 U/mg) was administered either subcutaneously or intracerebrally immediately after the single learning trial of the passive avoidance response. This time table was selected, since conventionally, memory consolidation processes — a term that denotes the input stage of memory — can be influenced by treatment given shortly after the learning trial and the effect can be measured 24 or 48 h later.

For subcutaneous administration, 5 μg AVP was dissolved in 0.5 ml saline, containing 1% of 0.01 N HCl. For intracerebral injection, 25 pg on either side (bilateral injection) or 50 pg (unilateral injection) of the peptide was dissolved in 0.5 μl saline, containing 0.1% of 0.01 N HCl. Control animals received the vehicle.

Statistical analysis

Data of passive avoidance behavior were analysed by Mann–Whitney's non-parametric ranking test. The two-tailed *t*-test was used for other calculations. A probability level of 0.05 or less was accepted as a significant difference.

RESULTS

Administration of arginine^8-vasopressin immediately after the learning trial in a dose of 5 μg s.c. resulted in significantly increased passive avoidance latencies in normal (sham-operated) rats as measured 24 and 48 h after treatment. Destruction of the dorsal noradrenergic bundle by bilateral microinjection of 6-OHDA did not influence avoidance behavior at the 24 h retention session. The 48 h avoidance latencies, however, were significantly lower than in the sham-operated control group. In the 6-OHDA-treated animals, postlearning administration of AVP did not influence avoidance behavior. The avoidance latencies of 6-OHDA-treated animals following vasopressin administration were in the range of saline-treated controls, and were significantly lower at both retention sessions than in sham-operated rats challenged with AVP (Fig. 1).

Destruction of the dorsal noradrenergic bundle decreased the open field ambulation and the number of rearings. Grooming activity and defecation were not influenced by the operation (Table I).

The effect of 6-OHDA treatment on catecholamine content in rats of the above experiments is presented in Table II. Microinjection of 6-OHDA in the dorsal noradrenergic bundle significantly decreased norepinephrine, but not the dopamine content in discrete brain regions (Table II).

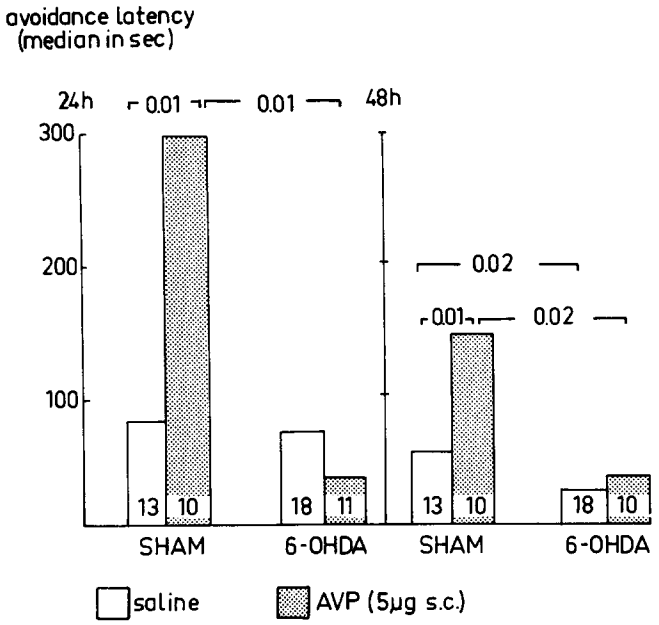

Fig. 1. Effect of selective chemical lesion of the dorsal noradrenergic bundle by 6-OHDA on the facilitation of passive avoidance behavior by postlearning administration of arg^8-vasopressin (AVP) as studied 24 and 48 h after the learning. Numbers in bars indicate the number of animals used. The significance of differences was calculated by means of Mann–Whitney test.

Injection of 6-OHDA in the nucleus accumbens or that of 5,6-DHT in the dorsal raphe nucleus did not prevent the facilitation of passive avoidance behavior, caused by postlearning administration of AVP. Although the 24 h avoidance latencies in both lesioned groups are slightly higher than in sham-operated controls, the 48 h avoidance latencies after vasopressin treatment are significantly longer in both lesioned groups than in saline-treated controls (Table III).

Postlearning local application of 25–25 pg AVP in the locus coeruleus did not influence passive avoidance behavior at either retention tests (Fig. 2).

TABLE I

Effect of bilateral microinjection of 6-OHDA in the dorsal noradrenergic bundle on open field activity

Activity was measured 20 days after operation. Data represent mean ± S. E.

	Sham operated (n = 13)	*6-OHDA (n = 12)*
Ambulation	49.3 ± 5.0	32.8 ± 5.3*
Rearing	9.8 ± 1.5	4.9 ± 0.9**
Grooming	4.7 ± 1.3	3.8 ± 1.2
Fecal boli	5.9 ± 0.6	5.7 ± 0.6

* $P < 0.05$; ** $P < 0.01$.

TABLE II

Effect of 6-OHDA, injected in the dorsal noradrenergic bundle, on the catecholamine content of discrete brain regions

Treatment entailed bilateral microinjection of 10 μg- 6-OHDA and determinations occurred 21 days after treatment. (n = 8–18 animals)

	Catecholamine content (pg/μg protein)		*% sham-operated*
	SHAM	*6-OHDA*	
Norepinephrine			
A_6 region	35.84 ± 5.17	15.52 ± 3.17*	43
Dentate gyrus	20.47 ± 2.17	11.46 ± 1.04*	56
Dopamine			
Nucleus accumbens	45.65 ± 5.45	40.65 ± 3.80	89
Nucleus caudatus	64.34 ± 13.87	70.08 ± 11.13	109

* $P < 0.05$.

A microinjection of 50 pg AVP in the midbrain dorsal raphe nucleus significantly facilitated passive avoidance behavior at the 24 h retention test. Microinjection of 5,6-DHT in the dorsal raphe nucleus did not influence passive avoidance behavior. In animals treated with 5,6-DHT, however, the effect of locally applied AVP was absent.

TABLE III

The effect of arg^8-vasopressin (5 μg s.c.), injected immediately after the learning trial, on retention of passive avoidance behavior in rats with 6-OHDA microinjection in the nucleus accumbens or 5,6-DHT microinjection in the dorsal raphe nucleus

Nucleus accumbens received a bilateral microinjection of 10–10 μg of 6-OHDA 10 days before determinations. Nucleus raphe dorsalis received a unilateral microinjection of 10–10 μg of 5,6-DHT 10 days before determinations.

	n	*Median avoidance latency (sec)*	
		24 h retention	*48 h retention*
Sham-operation			
Saline	9	68	60
AVP	11	300**	116*
Nucleus Accumbens (6-OHDA)			
Saline	6	130	91
AVP	10	185	184*
Nucleus Raphe Dorsalis (5,6-DHT)			
Saline	10	121	92
AVP	11	300	166*

* $P < 0.05$; ** $P < 0.01$.

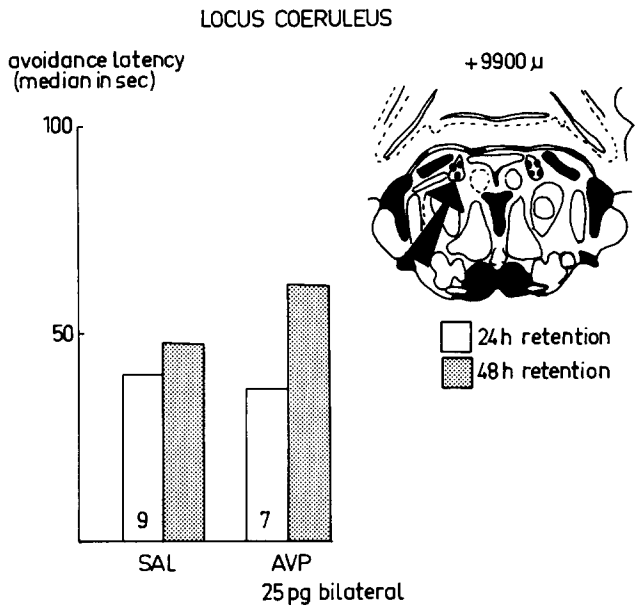

Fig. 2. Effect of postlearning local microinjection of arg^8-vasopressin (AVP) into the locus coeruleus on passive avoidance behavior.

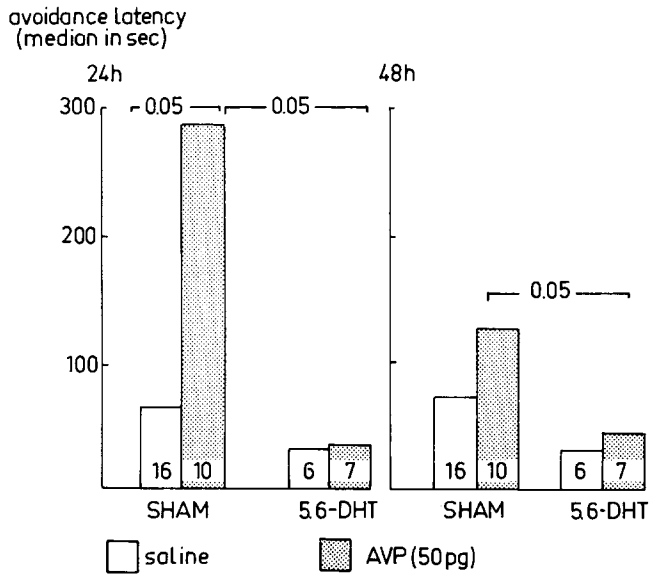

Fig. 3. Effect of selective chemical destruction of the dorsal raphe nucleus by 5,6-dihydroxytryptamine on the facilitation of passive avoidance behavior by postlearning microinjection of arg^8-vasopressin into the dorsal raphe nucleus. (See legend of Fig. 1.)

TABLE IV

Effect of 5,6-DHT on [^{3}H]serotonin uptake

	Uptake $\left(\frac{nCi \times 10^{-3}}{mg\ tissue}\right)$		
	Sham-operated	*5,6-DHT*	*% sham-operated*
Mesencephalon	368 ± 81	86 ± 43*	26
Dorsal hippocampus	297 ± 59	60 ± 26*	20

Determination carried out 21 days after operation. Data represent mean ± S. E. (n = 6)
* $P < 0.01$.

The avoidance latencies in these animals after microinjection of 50 pg AVP were not different from saline-treated, 5,6-DHT-lesioned controls and significantly shorter avoidance latencies were observed than in the sham-operated, AVP-treated rats (Fig. 3).

The effect of 5,6-DHT on [^{3}H]serotonin uptake is presented in Table IV. The neurotoxic compound significantly decreased 5-HT uptake in the mesencephalon and in the dorsal hippocampus.

Injection of 6-OHDA, in a dose of 10 μg into the dorsal raphe nucleus did not influence passive avoidance behavior. It prevented, however, the facilitation of passive avoidance response elicited by postlearning local application of AVP in the raphe nucleus (Fig. 4).

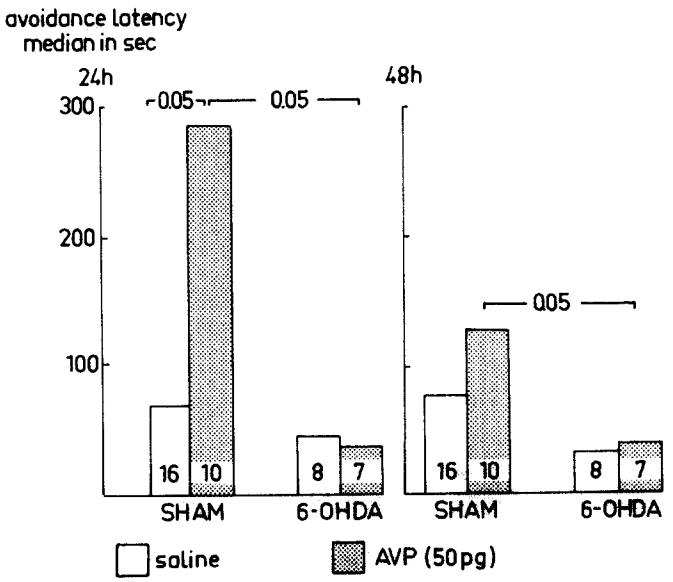

Fig. 4. Effect of microinjection of 6-OHDA into the dorsal raphe nucleus on the facilitation of passive avoidance behavior by postlearning microinjection of arg^8-vasopressin into the dorsal raphe nucleus. (See legend of Fig. 1.)

DISCUSSION

The present observations show that selective lesion of the dorsal noradrenergic bundle prevents the facilitatory action of vasopressin on a passive avoidance response. Thus, the data indicate that terminals of the dorsal noradrenergic bundle mediate the effect of vasopressin on memory consolidation processes.

Earlier studies have indicated that vasopressin facilitates consolidation (and retrieval) of memory[14]. It has also been shown that the neuropeptide modulates (in the majority of the brain regions it facilitates) cerebral catecholamine metabolism[21,22,36,37]. That parallel changes in behavior and catecholaminergic neurotransmission might be correlated has been suggested by the finding that a relatively low dose of repeatedly injected catecholamine synthesis inhibitor (α-methyl-*p*-tyrosine) prevented the effect of lys^8-vasopressin on avoidance behavior, leading to the assumption that either norepinephrine or dopamine might be involved in this effect of the peptide[21].

Local microinjection of neurotoxic compounds, such as 6-OHDA or 5,6-DHT into specific monoaminergic pathways of the brain provides a selective tool for analysis of the role of a particular neurotransmitter pathway[13,39]. Selective chemical destruction of the ascending dorsal noradrenergic bundle by 6-OHDA results in a depletion of noradrenaline levels[27], mainly in the forebrain. This is a consequence of the selective degeneration of noradrenergic neurons. No changes, however, occur in brain dopamine[34]. Our observation is in accord with these data, since destruction of the noradrenergic bundle resulted in a decreased noradrenaline level in the dorsal hippocampus and the locus coeruleus, while dopamine remained in the control range. The finding of the decreased ambulatory and rearing activities in lesioned animals supports these biochemical data, since ambulation and rearing have been shown to be correlated with noradrenergic[29] and grooming to be more related to dopaminergic activity in the brain[47]. Previous observations showed a similar decrease in ambulation and rearing following lesion of the locus coeruleus[3,15].

It is controversial in the literature whether the destruction of the dorsal noradrenergic bundle critically interferes with learning and memory. In our experience, the lesion slightly, but significantly impaired avoidance behavior at the 48 h retention session, supporting the importance of this pathway for long-term memory processes[5,10,11,18]. The fact, however, that the lesion did not exert dramatic influence on behavior rather substantiates the ideas of Mason and Iversen[26,27] that learning is possible in absence of forebrain noradrenaline.

It is clear, however, that the dorsal noradrenergic bundle should be intact for vasopressin-induced facilitation of memory consolidation to occur. Selective destruction of this pathway completely abolished the effect of arg^8-vasopressin — administered immediately after the learning trial — on the retention of a passive avoidance response. The question arose, if cell bodies or terminals of the noradrenergic bundle mediate the effect of vasopressin.

Local application of AVP in the locus coeruleus, the noradrenergic cell body region of the dorsal noradrenergic bundle, failed to affect memory consolidation. The same amount of AVP had already been shown to facilitate memory consolidation,

when injected into limbic-midbrain structures, such as the dorsal septum or the dentate gyrus[22]. Failure of AVP to modulate these processes from the A_6 region, therefore, may indicate that sensitive sites (putative peptidergic receptors?) are not localized on the cell bodies of these noradrenergic neurons.

The recent data, that lesion of the dorsal noradrenergic bundle prevents the effect of AVP on memory consolidation coupled with the finding that this bundle supplies noradrenergic inputs to all limbic-midbrain structures sensitive to memory effects of vasopressin investigated so far[12,16] suggest that terminals of the noradrenergic neurons are primarily involved. This notion is further supported by the observations on the role of the dorsal raphe nucleus in memory effects of vasopressin.

Evidence has accumulated that serotoninergic neurons of the dorsal raphe nucleus receive connections from the locus coeruleus and it has been postulated that noradrenergic terminals of these fibers relayed from the A_6 region modulate the activity of serotoninergic cell bodies[4,17,20,24,32,35]. Local application of AVP into this area, in an amount that appeared to be ineffective via intracerebroventricular route[8], also facilitated passive avoidance behavior. This facilitatory effect of the locally applied neuropeptide could be abolished by previous microinjection of 5,6-DHT but also by administration of 6-OHDA into this area. 5,6-DHT causes a selective degeneration of the serotoninergic neurons[13], and also in our experience it decreased 5-HT uptake in the mesencephalon and in the dorsal hippocampus. It is clear, therefore that the serotoninergic neurons are involved in the memory effect of locally applied AVP in the dorsal raphe nucleus. Since however, microinjection of 6-OHDA into the dorsal raphe nucleus also prevented the memory effect of the locally applied neuropeptide one might assume that the neuropeptide primarily affected noradrenergic nerve terminals in the raphe area. According to this hypothesis, the serotoninergic system represents the output pathway of the action of locally applied neuropeptide and its involvement is secondary, due to a primary interaction of the peptide with noradrenergic terminals and to an influence of these terminals on serotoninergic perikarya. The exact role of the serotoninergic system, however, remains to be solved (Fig. 5).

The role of the dorsal noradrenergic bundle seems to be a primary and selective one. Neither 6-OHDA-induced destruction of the nucleus accumbens, which is a part of the mesolimbic dopaminergic system, nor the 5,6-DHT-induced lesion of the dorsal raphe nucleus, the major source of the ascending serotoninergic system, interfered with the effect of AVP, administered *systemically*. That neurochemical lesion of the dorsal raphe nucleus prevented the effect of AVP applied locally into the raphe area, but not if the peptide was administered systemically, is in keeping with the role of noradrenergic terminals. After systemic administration of the neuropeptide, vasopressin might have influenced intact noradrenergic terminals and in turn facilitated avoidance behavior (Fig. 5).

Exogenous vasopressin was used to study the involvement of noradrenergic terminals in peptide-induced alterations of memory processes. Vasopressin, however, is physiologically present in the cerebrospinal fluid and in extrahypothalamic magnocellular nuclei and projecting to all limbic-midbrain structures sensitive to memory

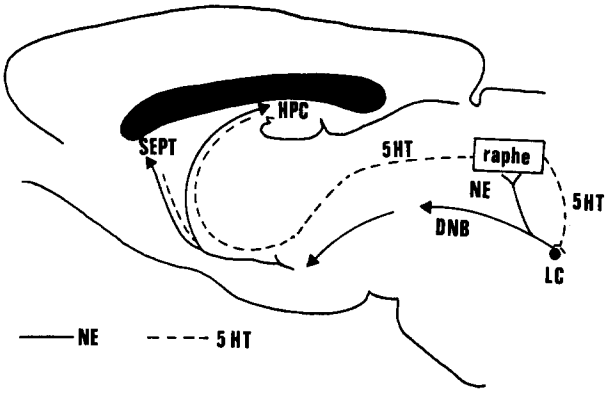

Fig. 5. Schematic illustration of neurotransmitter pathways involved in the action of vasopressin. Abbreviations: HPC, hippocampus; sept, septum; LC, locus coeruleus; DNB, dorsal noradrenergic bundle; NE, norepinephrine; 5HT, serotonin.

effects of vasopressin[9,46]. This peptidergic network is absent in rats homozygous for hereditary hypothalamic diabetes insipidus[9] (Brattleboro strain). Recent studies revealed that vasopressin is physiologically involved in memory processes. Hereditary hypothalamic diabetes insipidus animals exhibit severe memory deficits[7]. Furthermore, neutralization of vasopressin in the brain by specific antiserum impairs memory formation in normal rats[43]. In addition, the pattern of catecholamine disappearance in specific brain regions in diabetes insipidus animals[44] and in normal rats after vasopressin antiserum (unpublished observation) is opposite to that of normal rats after vasopressin treatment[37,41]. On the basis of these and our present data, therefore, it is logical to assume that extrahypothalamic vasopressinergic fibers and/or vasopressin present physiologically in the cerebrospinal fluid modulate the ongoing activity of noradrenergic neurons at the presynaptic level.

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