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Behavioral Effects of Intracerebroventricularly Administered Neurohypophyseal Hormone Analogs in Mice

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MEISENBERG, G. AND W. H. SIMMONS. *Behavioral effects of intracerebroventricularly administered neurohypophyseal hormone analogs in mice.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 819–825, 1982.—The neurohypophyseal hormones oxytocin and vasopressin evoke characteristic behavioral changes after intracerebroventricular injection in mice. These include the induction of excessive grooming and scratching in an unrestricted environment and of escape behavior in stressful situations. The structure-activity relations of 33 neurohypophyseal hormones and hormone analogs in the induction of these behavioral changes were examined. The results demonstrate that these effects generally parallel the vasoconstrictory effects of the peptides. It is suggested that the behavioral effects are mediated by a receptor that is closely related biochemically to the vasopressin receptors in blood vessels. They are not related to the long-term effects of neurohypophyseal hormones on learned behavior. A competitive antagonist of the short-term behavioral effects of these peptides is described. This analog also antagonizes some of the hormonal effects of vasopressin and related peptides.

Oxytocin Vasopressin Analogs Antagonist Structure-activity relations

THERE are several behavioral effects of vasopressin-like peptides in animals including well described effects on memory processes and avoidance behavior [2, 42, 58, 59], attenuation of puromycin-induced amnesia [32,53], alteration of the hippocampal theta rhythm [2], enhancement of the formation of tolerance to effects of morphine and ethanol [28,31], and suppression of heroin self-administration [40]. Intracranial self-stimulation is enhanced by oxytocin and suppressed by vasopressin [43,44].

These effects have some features in common: They are elicited by picogram amounts of the hormones after central application [43, 44, 59], they can be observed after systemic application as well [2, 44, 58, 59], and oxytocin and vasopressin frequently act in opposite directions [2, 42, 43, 44], at least if the peptides are applied centrally [2]. All of these effects are of long duration, with some of them being observable days or even weeks after treatment [2, 59, 60]. The structure-activity relationships appear to be unrelated to those for the hormonal effects of these peptides [53, 55, 59].

A different type of behavioral effect has been described more recently: After intracerebroventricular injections in mice, the peptides induce a syndrome of hyperactivity with excessive grooming, scratching, foraging and vocalization [13, 14, 16, 37, 38, 41]. These effects have been shown to be dependent on the enviromental conditions: The unrestrained mouse develops excessive scratching and grooming behavior, whereas in stressful situations escape-directed activity is markedly stimulated [37]. These effects are of short duration: They subside within one hour and appear to be completely reversible [37]. The effects of different neurohypophyseal hormones cannot easily be distinguished from each other behaviorally [37] or pharmacologically [38], suggesting a common mechanism of action. Systemically administered neurohypophyseal hormones are not capable of inducing the characteristic behavioral alterations [13,37]. The mechanisms and the physiological significance of these effects are unknown. To obtain information on the mechanism of action, we have investigated the behavioral effects of neurohypophyseal hormone analogs and compared them with the hormonal effects described in the literature.

METHOD

Peptides

[8-Arginine] vasopressin (367 IU/mg), [8-lysine] vasopressin (231 IU/mg), oxytocin (500 IU/mg), and [8-arginine] vasotocin were obtained from Sigma Chemical Co. Isotocin was obtained from Serva. [Des-9-glycinamide, 8-lysine] vasopressin was prepared from [8-lysinie] vasopressin by tryptic digestion [37]. N-acetyl-[2-(0-methyl) tyrosine, 8-arginine] vasopressin and [2-(0-methyl)tyrosine, 8-arginine] vasopressin were the generous gifts of Dr. David A. Jones, Searle Research and Development, Chicago, IL and Dr. Wilbur H. Sawyer, Columbia University, NY. All other peptides were available at the University of Illinois at the Medical Center, Chicago, IL, and were synthesized in the laboratories of the late Dr. Roderich Walter and the late Dr. Vincent du Vigneaud, Cornell University (see Table 1). The actual source of each peptide and the published biological activities are given in Table 2.

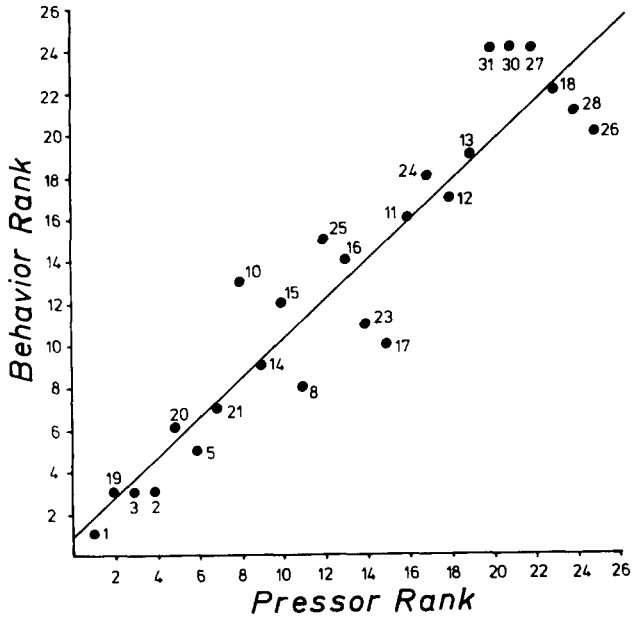

FIG. 1. Relationship between behavior-altering potency and rat pressor potency. Neurohypophyseal hormones and analogs in Table 2 tested for both behavior-altering and rat pressor activities were ranked according to their potency in each assay. Analogs whose activities were less than the lowest dose tested were group together at the lowest rank.

Animals

Male albino mice of the strain CF1/W68, weighing 25–30 g, were obtained from Fa. Winkelmann & Co in Paderborn, W. Germany. The animals were kept in groups of 10–15 mice per cage at a constant temperature of 22–23°C in a 12× 12 hr light-dark cycle.

Injections

The peptides were injected intracerebroventricularly in light ether anesthesia in a volume of 20 μl 150 mM NaCl. Animals injected with saline alone served as controls. Details of the procedure have been described elsewhere [37]. In the case of the antagonist experiments, agonist and antagonist were administered in a single injection containing both agents.

Biological Testing

The activity unit used for the quantitation of the behavior-altering effect has been described elsewhere [37]. In short, the mouse was placed into a Plexiglas cage after the injection, and 2–5 minutes after the injection the incidence of excessive scratching (scratching for 10 seconds or more) was determined. One unit is that amount of a peptide which would be sufficient to induce excessive scratching in 50% out of ten thousand mice. The dose sufficient to induce excessive scratching in 50% of the mice was determined by testing serial dilutions of the peptides in at least 10–15 mice each.

The tube test has been described earlier [37]. In short, 6 minutes after the injection the mouse was pushed into a Plexiglas tube, 3.6×30 cm, and the tube was placed upright. After 2 minutes, immobility and efforts to escape were recorded cumulatively for 4 minutes in seconds per test session.

The "scratching-mouse test" was performed as described previously [37]. In short, the mouse was placed into a plastic cage, and immobility and scratching + grooming were recorded cumulatively for 5 minutes 6–11 minutes after the injection.

TABLE 1

STRUCTURE OF NEUROHYPOPHYSEAL HORMONES

1 2 3 4 5 6 7 8 9

Cys-Tyr-X-Y-Asn-Cys-Pro-Z-Gly-NH_2

S–S

Peptide	X	Y	Z
[8-arginine]vasopressin	Phe	Gln	Arg
[8-lysine]vasopressin	Phe	Gln	Lys
[8-arginine]vasotocin	Ile	Gln	Arg
oxytocin	Ile	Gln	Leu
isotocin	Ile	Ser	Ile

RESULTS

Structure-Activity Relationships

Table 2 shows the behavior-altering potency of 33 different neurohypophyseal hormones and analogs as well as the peripheral hormonal activities of these peptides. The behavior-altering potency markedly paralleled the potency of the peptides in the rat blood pressure assay. Figure 1 shows that the behavior-altering and pressor potencies of the analogs were highly correlated, giving $\rho=0.94$ using the Spearman rank-order correlation method [7]. Notable anomalies were the 7-glycine analogs of oxytocin and deamino oxytocin (30 and 31) and isotocin (27), which were completely inactive in the behavioral test despite having some pressor activity [24,53] and [2-phenlyalanine, 8-lysine] vasopressin (10) which was less active than could be predicted from its high pressor activity [35]. Nevertheless, the correlation between behavior-altering and pressor potencies was significantly better ($p<0.001$) than the correlation between behavior-altering and antidiuretic potencies ($\rho=0.75$). Analogs having a 4-aminobutyric acid (14, 15, 16) or a 7-(3,4-dehydroproline) (19, 20, 21, 22) demonstrated a clear dissociation of behavior-altering and antidiuretic effects. This was particularly evident with deamino [2-phenylalanine, 7-(3,4-dehydroproline), 8-arginine] vasopressin (22) which has extraordinarily high antidiuretic activity (13000 IU/mg) [5], but quite modest behavior-altering activity (7.3 units/mg). There was also an extremely poor correlation between behavior-altering potency and rat uterotonic potency ($\rho=-0.05$) or avian depressor potency ($\rho=-0.22$).

No analog had a higher behavior-altering potency than the natural vasopressins. Two other natural peptides recently isolated from bovine neurohypophysis [23], valyl-aspartyl- and alanyl-glycyl-[8-arginine] vasopressin (6 and 7), exhibit strong natriuretic effects but little pressor activity [11,12]. Both of these peptides had low behavioral activities suggesting that they are not enzymatically converted to [8-arginine]

TABLE 2

BIOLOGICAL EFFECTS OF NEUROHYPOPHYSEAL HORMONES AND ANALOGS

Peptide	Mouse Behavior*	Rat Antidiuretic*	Rat Pressor*	Avian Depressor*	Rat Uterotonic*
1 [8-arginine]vasopressin	220	503 [36]	487 [36]	100 [36]	12 [36]
2 [8-lysine]vasopressin	180	203 [30]	243 [30]	48 [30]	4.8 [30]
3 [8-arginine]vasotocin	180	195 [18]	245 [18]	150 [4]	125 [18]
4 [des-9-glycinamide,8-lysine] vasopressin	<2.0	2.4 [53]	0.05 [53]	inhib. [53]	0.22 [53]
5 deamino[8-lysine]vasopressin	110	301 [30]	126 [30]	48 [30]	12 [30]
6 valyl-aspartyl-[8-arginine] vasopressin	<0.01	—	—	—	—
7 alanyl-glycyl-[8-arginine] vasopressin	19	—	—	—	—
8 [2-(0-methyl)tyrosine, 8-arginine]vasopressin	19	386 [29]	9.7 [29]	—	inhib. [29]
9 N-acetyl-[2-(0-methyl)tyrosine, 8-arginine]vasopressin	inhib.	0.026 [29]	inhib. [29] $pA_2=7.18$	—	inhib. [29] $pA_2=7.29$ (no Mg^{++}) $pA_2=6.73$ (Mg^{++})
10 [2-phenylalanine,8-lysine] vasopressin	3.1	21 [3]	50–60 [35]	0.5 [35]	<0.1 [35]
11 deamino[2-isoleucine,8-lysine] vasopressin	1.5	3.0 [26]	1.5 [26]	1.0 [26]	<0.1 [26]
12 [4-leucine,8-lysine]vasopressin	1.3	1–2 [17]	1.33 [17]	inhib. [17]	inhib. [17]
13 [4-N^5,N^5-dimethylglutamine, 8-lysine]vasopressin	0.65	1.88 [47]	1.27 [47]	<0.1 [47]	<0.05 [47]
14 [4-aminobutyric acid,8-arginine] vasopressin	14	760 [22]	38 [22]	25 [22]	5 [22]
15 deamino[4-aminobutyric acid, 8-arginine]vasopressin	3.6	1020 [22]	10.7 [22]	9.9 [22]	3.3 [22]
16 deamino[4-aminobutyric acid,8-lysine] vasopressin	2.9	729 [21]	3.5 [21]	12.6 [21]	1.51 [21]
17 [5-(N^4,N^4-dimethylasparagine,8-lysine] vasopressin	7.7	5.5 [45]	2.55 [45]	0.39 [45]	<0.05 [45]
18 deamino[5-alanine,8-lysine] vasopressin	0.008	0.05 [21]	0.015 [21]	<0.01 [21]	<0.002 [21]
19 [7-(3,4-dehydroproline),8-arginine] vasopressin	180	1260 [5]	255 [5]	40.6 [5]	76.9 [5]
20 deamino[7-(3,4-dehydroproline), 8-arginine]vasopressin	100	4134 [5]	240 [5]	345 [5]	69 [5]
21 [2-phenylalanine,7-(3,4-dehydro-proline),8-arginine]vasopressin	40	1541 [5]	63.8 [5]	4.2 [5]	0.8 [5]
22 deamino[2-phenylalanine,7-(3,4-dehydroproline),8-arginine]vasopressin	7.3	13000 [5]	—	2.9 [5]	6.0 [5]
23 oxytocin	6.8	2.7 [9]	3.1 [9]	507 [9]	546 [10]
24 deamino oxytocin	1.1	19 [19]	1.44 [19]	975 [19]	803 [19]
25 deamino[8-alanine]vasopressin	2.0	—	4.6 [52]	47 [52]	25 [52]
26 [4-valine]oxytocin	0.02	—	0 [51]	240 [51]	140 [51]
27 isotocin	<0.001	0.18 [24]	0.06 [24]	320 [24]	150 [24]
28 deamino[4-norvaline]oxytocin	<0.01	0.03 [20]	<0.001 [20]	128 [20]	56 [20]
29 5-(N^4,N^4-dimethylasparagine) oxytocin	0.05	—	—	9 [56]	4.6 [56]
30 [7-glycine]oxytocin	<0.01	<0.01 [54]	0.3 [54]	5.3 [54]	65 [54]
31 deamino[7-glycine]oxytocin	<0.01	0.062 [54]	0.5 [54]	17 [54]	355 [54]
32 deamino oxytocinoic acid dimethylamide	0.015	—	—	0 [49]	16 [49]
33 Z-prolyl-leucyl-glycinamide	<0.005	—	—	—	—

*All data in units/mg.

TABLE 3
EFFECTS OF SOME ANALOGS IN THE TUBE TEST

Peptide	Dose	N*	Immobility†	Escape-activity†
NaCl		(16)	219.1 ± 7.9	1.9 ± 1.0
1 [8-arginine]vasopressin	1 ng	(8)	212.8 ± 9.9	1.5 ± 1.2
	10 ng	(8)	180.3 ± 10.5§	25.8 ± 11.3‡
	100 ng	(8)	48.4 ± 9.6¶	96.5 ± 18.3¶
17 [5-(N^4,N^4-dimethylasparagine),	10 ng	(8)	225.6 ± 6.2	0.3 ± 0.3
8-lysine]vasopressin	100 ng	(8)	164.4 ± 14.1§	24.8 ± 13.8§
	1 μg	(8)	56.3 ± 12.8¶	79.8 ± 18.5¶
22 deamino[2-phenylalanine,7-	10 ng	(8)	209.8 ± 9.9	9.3 ± 4.7‡
(3,4-dehydroproline),8-	100 ng	(8)	104.4 ± 15.5¶	52.6 ± 11.2¶
arginine]vasopressin	1 μg	(8)	18.0 ± 6.6¶	86.3 ± 11.2¶
23 oxytocin	20 ng	(8)	199.8 ± 12.0	3.8 ± 1.4
	200 ng	(8)	138.8 ± 10.2¶	59.8 ± 11.1¶
	2 μg	(8)	60.8 ± 8.8¶	105.6 ± 15.5¶
24 deamino oxytocin	250 ng	(8)	164.0 ± 9.8¶	34.8 ± 10.1§
	2.5 μg	(8)	51.5 ± 7.7¶	95.0 ± 15.6¶
27 isotocin	50 μg	(8)	223.4 ± 11.1	0.4 ± 0.4
30 [7-glycine]oxytocin	5 μg	(8)	213.4 ± 5.3	3.3 ± 3.3

*Number of mice per group.
†In seconds per test session, mean ± S.E.M.
‡$p<0.1$; §$p<0.01$; ¶$p<0.001$; Mann-Whitney's U-test.

vasopressin in the brain to any great extent following intraventricular injection.

There were no analogs which exhibited a markedly enhanced selectivity for the behavioral effect. For example, the highest behavioral/rat pressor ratios, compared with LVP, were 4.1 in [5-(N^4, N^4-dimethylasparagine), 8-lysine] vasopressin (17) and 3.0 in oxytocin (23). The highest behavioral/antidiuretic ratios were 2.8 in oxytocin and 1.6 in [5-(N^4, N^4-dimethylasparagine), 8-lysine] vasopressin.

Other Short-Term Behavioral Effects

Table 3 shows the effects of some analogs in the tube test: The activity of the analogs in this test largely paralleled their potency in the induction of excessive scratching behavior that has been used for the definition of the activity unit. Only slight dissociations were observed. This suggests that the different short-term behavioral effects of neurohypophyseal hormones in mice are mediated by receptors with very similar biochemical properties.

Antagonism

One of the analogs, N-acetyl-[2-(0-methyl) tyrosine, 8-arginine] vasopressin (9) (Ac-Tyr(Me)AVP), was found to be an antagonist of the behavioral effects of neurohypophyseal hormones. This analog is also an antagonist of the rat pressor activity of vasopressin but a weak antidiuretic agonist [29]. Figures 2 and 3 show the effects of injection of 5 μg Ac-Tyr(Me)AVP along with different doses of [8-arginine] vasotocin (AVT). Antagonism of the behavioral effects could be demonstrated only at the lower and intermediate doses of AVT. The maximal effect of AVT was not reduced suggesting that the analog is acting as a competitive inhibitor.

The antagonist activity of Ac-Tyr(Me)AVP in the behavioral assay was weak, requiring microgram quantities to reduce the effect of 5 or 10 ng of AVT. However, on the basis of its antivasopressor pA_2 value (7.18) [29], it is expected that at least a 100–200-fold molar excess of Ac-Tyr(Me)AVP would also be required to partially block the pressor response to an intravenous challenge dose of AVP of 25–50 ng/kg [34]. Thus it appears that receptors in the brain and peripheral vasculature responsible for the observed biological effects may behave similarly towards the agonist and antagonist.

Although Ac-Tyr(Me)AVP is an antagonist of the rat oxytocic (uterotonic) activity of neurohypophyseal hormones as well, other oxytocic antagonists including [2-(0-methyl)tyrosine,8-arginine] vasopressin (8) and [4-leucine,8-lysine] vasopressin (12) did not antagonize the behavior-altering effects of AVT. Indeed, removal of the acetyl group from Ac-Tyr(Me)AVP changed the peptide from an antagonist to a behavior-altering agonist as well as a pressor agonist (8). None of the other analogs demonstrated antagonist activity.

DISCUSSION

The structure-activity relations of neurohypophyseal hormone analogs suggest that their short-term behavioral effects are mediated by a receptor closely resembling biochemically the vasopressin receptors in peripheral organs, especially those that mediate the pressor effect in rats. An action of centrally administered neurohypophyseal hormones on peripheral blood vessels, however, it is not likely to be involved in the induction of the behavioral alterations, since the peripherally administered hormones are inactive in this respect [13,37]. An action on cerebral blood vessels, however, appears to be possible: The smooth muscles surrounding the cerebral arterioles and venules are located inside the blood-brain barrier [6] and should be reached by centrally, but not by systemically applied peptides.

Little is known about the sensitivity of cerebral blood vessels to centrally applied vasopressin. In anesthetized rhesus monkeys, centrally applied vasopressin at rather high

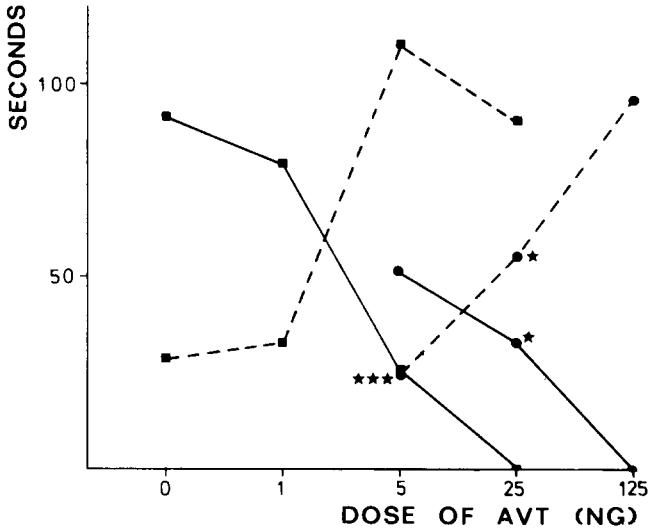

FIG. 2. Antagonism of behavioral effects of various doses of [8-arginine] vasotocin (AVT) in the "scratching test" by 5 μg of N-acetyl-[2-(0-methyl) tyrosine, 8-arginine] vasopressin. Each group contains 8 mice. ■—■ immobility after AVT alone. ●—● immobility after AVT + antagonist. ■---■ grooming + scratching after AVT alone. ●---● grooming + scratching after AVT + antagonist. ★★★$p<0.001$; Mann-Whitney's U-test.

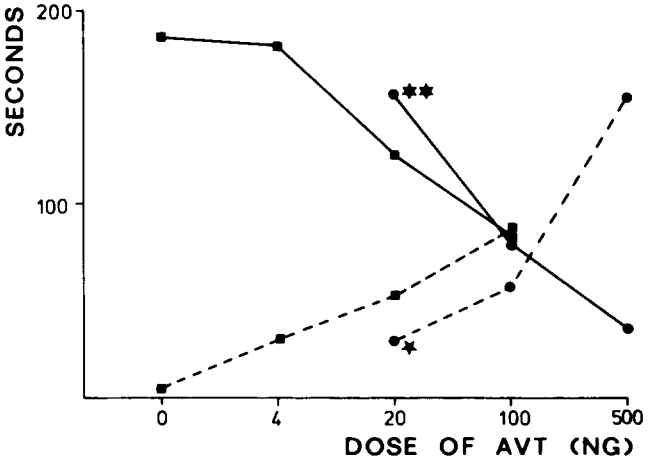

FIG. 3. Antagonism of behavioral effects of various doses of [8-arginine] vasotocin (AVT) in the tube test by 5 μg of N-acetyl-[2-(0-methyl) tyrosine, 8-arginine] vasopressin. Each group contains 8 mice. ■—■ immobility after AVT alone. ●—● immobility after AVT + antagonist. ■---■ escape-directed activity after AVT alone. ●---● escape-directed activity after AVT + antagonist. ★★$p<0.01$; Mann-Whitney's U-test.

doses causes enhancement of brain water permeability [39], although the cyclic-AMP content of cerebral microvessels from rat brain is not altered by vasopressin [27]. No change in overall cerebral blood flow was observed in monkeys after centrally applied vasopressin [39], and topical application of vasopressin and oxytocin to the surface of exposed rat brain failed to change the diameter of pial terminal arterioles [33]. These results do not preclude the possibility that vasopressin may induce alterations in regional cerebral blood flow since different blood vessels have been observed to exhibit different sensitivities to neurohypophyseal hormones even in the same species [1]. Other centrally applied vasoconstrictory agents such as ergotamine, amphetamine, adrenaline, prostaglandin $F_{2\alpha}$, and angiotensin are not capable of inducing behavioral alterations similar to those observed after application of neurohypophyseal hormones [13, 25, 38]. Therefore, a mediation of the behavioral effects by a direct neuronal mechanism appears to be more likely. The question as to whether the receptors mediating these effects are located at cerebral blood vessels or at neuronal structures cannot presently be answered.

While centrally administered vasopressin has been shown to induce bradycardia [50], it is unlikely that this effect is involved in the observed behavioral changes. The structure-activity-relationships of this centrally-mediated cardiovascular effect have not yet been investigated.

There appears to be no correlation betweeen the short-term behavioral effects and the effects on learned behavior reported previously [14, 53, 55]. The most prominent difference is the very low activity of [des-9-glycinamide-8-lysine] vasopressin in the induction of the acute behavioral effects. This peptide has a high vasopressin-like activity in a variety of experiments involving learned behavior [31, 32, 40, 60]. Even much shorter fragments of neurohypophyseal hormones have been shown to be active against retrograde amnesia [53] and extinction of pole-jumping avoidance behavior [55,59].

The physiological significance of the short-term behavioral effects of neurohypophyseal hormones is unknown. The presence of a specific receptor and the high potencies of the naturally occurring neurohypophyseal hormones support a role for these peptides for the regulation of animal behavior. Antagonists such as N-acetyl-[2-(0-methyl) tyrosine, 8-arginine] vasopressin, that specifically block this receptor, may prove to be useful for the elucidation of the physiological role of these effects: The antagonist would suppress the effects not only of centrally applied neurohypophyseal hormones and analogs, but of the endogenous peptides as well. Vasopressin and oxytocin are normal constituents of the cerebrospinal fluid [15], and nerve fibers containing oxytocin, vasopressin or neurophysin are present not only in the hypothalamus, but in other brain regions as well [8, 46, 48, 57]. Therefore, it is possible that extracellular concentrations of the peptides sufficient to stimulate the receptors responsible for the short-term behavioral effects can be reached by local release.

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