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Interactive effects of neurohypophyseal neuropeptides with receptor antagonists on passive avoidance behavior: Mediation by a cerebral neurohypophyseal hormone receptor?

(vasopressin/oxytocin/[4-pyroglutamic acid,6-cystine,8-arginine]vasopressin-(4–8)/[4-pyroglutamic acid,6-cystine]oxytocin-(4–8)/vasopressin and oxytocin antagonists)

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ABSTRACT **The neurohypophyseal neuropeptides (Arg^8)-vasopressin (AVP) and [$pGlu^4$,Cyt^6]AVP-(4–8) (where pGlu is pyroglutamic acid and Cyt is cystine) facilitate the retention of one-trial-learning passive avoidance behavior in rats when administered into the cerebral ventricle immediately after the learning trial. The fragment [$pGlu^4$,Cyt^6]AVP-(4–8) was considerably more effective than AVP. Oxytocin (OXT) and [$pGlu^4$,Cyt^6]OXT-(4–8) have the opposite effect and attenuate passive avoidance behavior also when administered into the cerebral ventricle after the learning trial. Again the fragment was more active than the parent molecule. The ancient arginine-containing neurohypophyseal hormone vasotocin in "high" doses (10 ng) had a vasopressin-like effect and in "low" doses (0.1 ng) had an OXT-like effect on passive avoidance behavior. Because both vasopressinergic (V_1) and oxytocinergic receptors have been demonstrated in the central nervous system, we asked whether specific antagonists of the V_1, V_2, and OXT receptor could antagonize the effects of these neuropeptides on passive avoidance behavior. The three antagonists were approximately equally active in blocking the effect of vasopressin, whereas the fragment [$pGlu^4$]AVP-(4–8) and the high dose of vasotocin were more readily blocked by the OXT antagonist. The attenuating effect of OXT, the fragment [$pGlu^4$,Cyt^6]OXT-(4–8), and the low dose of vasotocin was markedly reduced by the OXT antagonist. This effect could also be reduced by pretreatment with the V_1 antagonist but not with the V_2 antagonist. These results suggest the existence of a separate neurohypophyseal hormone receptor complex in the brain affecting memory processes that differs from the peripheral V_1, V_2, and OXT receptor.**

Numerous reports suggest profound effects of exogenously administered (1, 2) and centrally released (for review, see ref. 3) neurohypophyseal neuropeptides on processing of newly acquired information. Recently, vasopressinergic (4–6) and oxytocinergic (6–8) binding sites have been described in the central nervous system. Evidence has accumulated that the larger part of specific vasopressinergic binding sites in the limbic system—the brain areas mediating the action of neurohypophyseal neuropeptides on learning/memory processes—have a ligand specificity that resembles that of peripheral vasopressinergic receptors of the V_1 type. In agreement with this suggestion is the behavioral observation (9, 10) that the V_1 vasopressinergic antagonist d(CH_2)$_5$-[Tyr(Me)^2,Arg^8]vasopressin [where d(CH_2)$_5$ represents β-mercapto-β,β-cyclopentamethylenepropionic acid] prevented the effect of [Arg^8]vasopressin (AVP) on passive avoidance behavior. This effect was found after either s.c. or intracerebroventricular (i.c.v.) challenge (11). The V_1 vasopressinergic antagonist was also effective in preventing the action of [$pGlu^4$,Cyt^6]AVP-(4–8) (where pGlu is pyroglutamic acid and Cyt is cystine), a more selective behaviorally active putative endogenous metabolite of AVP (12). Because [$pGlu^4$,Cyt^6]AVP-(4–8) had no effect on blood pressure, diuresis, and body temperature, these data indicated that the receptors involved in the action of AVP on behavior are clearly separated from those involved in the pressor response (11, 13).

More recent data, however, raised the possibility that the involvement of various neurohypophyseal neuropeptide-receptor types might be more complex than originally suggested. Accordingly, V_2-type vasopressinergic receptors have been suggested to be present in the brain (14). Moreover, oxytocin (OXT) receptors have been shown to bind AVP and vasotocin as efficiently as OXT. Thus cerebral OXT receptors discriminate rather poorly between AVP, vasotocin, and OXT (6, 8).

These data prompted us to investigate the interaction of AVP, OXT, and the behaviorally active fragments [$pGlu^4$,Cyt^6]AVP-(4–8) and [$pGlu^4$,Cyt^6]OXT-(4–8) with selective V_1, V_2 vasopressinergic, and oxytocinergic receptor antagonists on the retention of passive avoidance behavior.

METHODS

Experimental Animals. Male Wistar rats of an inbred strain (Harlan CBP, Zeist, The Netherlands) were used. The weight of the experimental animals was 160 ± 20 g. All animals had free access to food and drinking water and were kept on a controlled illumination schedule with lights on between 6 a.m. and 8 p.m.

Operation. For intracerebral cannulation rats were equipped with a polyethylene cannula implanted into the lateral cerebral ventricle. Cannulation was performed under fluanisone/fentanyl/Hypnorm anesthesia. After the operation, rats were housed in separate cages and allowed to recover from the operation for 4 days.

Passive Avoidance Behavior. Animals were trained in a one-trial-learning passive avoidance test, as described in detail (15). The experimental apparatus consisted of an illuminated platform attached to a large compartment. Rats

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Abbreviations: AVP, [Arg^8]vasopressin; OXT, oxytocin; pGlu, pyroglutamic acid; Cyt, cystine; d(CH_2)$_5$-, β-mercapto-β,β-cyclopentamethylenepropionic acid; AAVP(V_1), antagonist d(CH_2)$_5$-[Tyr(Me)^2]AVP for V_1-type AVP receptor; AAVP(V_2), antagonist d(CH_2)$_5$[D-Ile^2,Ile^4]AVP for V_2-type AVP receptor; AOXT, antagonist Des-Gly, NH_2-dCH_2)$_5$[Tyr(Me^2),Thr^4,Orn^8]vasotocin (where Orn is ornithine) for OXT receptor; i.c.v., intracerebroventricular; Sal, saline.

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were placed on the platform and allowed to enter the dark compartment. After three more trials on the following day, an unavoidable scrambled foot shock of 0.25 mA for 2 sec was delivered through the grid floor of the dark compartment (learning trial). Retention of passive avoidance behavior was measured 24 and 48 hr after the learning trial—i.e., animals were placed on the platform, and the latency to enter the dark compartment was measured up to a maximum of 300 sec. To assess the attenuating effect of OXT, vasotocin, and the fragment [pGlu^4,Cyt^6]OXT-(4–8) on passive avoidance behavior, rats were exposed to 0.5 mA for 2 sec. All treatments were given immediately after the learning trial.

Treatment. All treatments were given i.c.v. in a blind manner, the code being broken only after data analysis. The antagonist or the vehicle for the antagonist was injected immediately after the learning trial. The neurohypophyseal peptides or the vehicles for these peptides were injected 30 min later. The following substances were synthesized by J. W. van Nispen (Organon, Oss, The Netherlands): AVP (Org 5477, HPLC purity 93.7%); OXT (Org 4882 D, HPLC purity 99%), [pGlu^4,Cyt^6]AVP-(4–8) (Org 30.337, HPLC purity 98.5%), and [pGlu^4,Cyt^6]OXT-(4–8) (Org 30.338A, HPLC purity 96%). The antagonists d(CH$_2$)$_5$[Tyr(Me)^2]AVP [AAVP(V$_1$)] for the V$_1$ type and d(CH$_2$)$_5$[D-Ile^2,Ile^4]AVP [AAVP(V$_2$)] for the V$_2$ type vasopressinergic receptors and Des-Gly,(NH$_2$)^9-d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Orn^8]vasotocin (where Orn is ornithine) (AOXT) for the OXT receptors, were donated by Maurice Manning (Medical College of Ohio, Toledo). The substances were dissolved in saline (Sal) immediately before use and injected i.c.v. A volume of 1 μl was injected at a slow infusion rate. The antagonist or the control solution was administered immediately after the learning trial followed 30 min later by agonist or placebo. The agonistic and/or antagonistic effects of the antagonists for blood pressure, antidiuresis, and uterus contraction and V$_1$, V$_2$, and OXT receptor binding affinities are summarized in Table 1.

Histological Control. Localization of the cannula tip was determined at the end of the experiment by injection of Evans blue.

Statistical Analysis. Differences in passive avoidance latencies were analyzed by the Mann–Whitney nonparametric ranking test.

RESULTS

Table 2 summarizes the effects of graded doses of the receptor antagonist when injected alone. Neither AAVP(V$_1$), AAVP(V$_2$), nor AOXT in the doses used significantly affected the retention of passive avoidance behavior. The effect of postlearning AVP treatment and the interaction of AVP with various doses of the receptor antagonists is also summarized in Table 2. Statistical analysis revealed significant differences in the 24-hr and 48-hr avoidance latencies among the various treatment groups. AVP in a dose of 1 ng very significantly facilitated retention of passive avoidance behavior at both 24- and 48-hr retention sessions. Pretreatment with lower doses of the receptor antagonists reduced the AVP effect. Surprisingly, the 0.1-ng, but not the 0.3-ng, dose of AAVP(V$_1$) significantly attenuated the effect at both the 24-hr and 48-hr retention tests. The AOXT markedly attenuated AVP-induced passive avoidance behavior in a dose of 0.3 ng at the 24-hr retention and 48-hr retention tests. AAVP(V$_2$) also attenuated the AVP effect in a dose of 0.3 ng per rat. Pretreatment (i.c.v.) with 1 ng of AAVP(V$_1$), AAVP(V$_2$), or AOXT effectively blocked the action of i.c.v. AVP treatment on passive avoidance behavior.

Table 2. Interaction of 1 ng of AVP with graded doses of the various neurohypophyseal receptor antagonists on the retention of passive avoidance behavior

Treatment	*n*	Passive avoidance latency*, sec: 24-hr retention		48-hr retention	
Sal					
+ Sal	16	38	(9–66)	14	(5–45)
+ AVP	25	186	(88–300)†	138	(79–300)†
AAVP(V$_1$)					
0.1 ng + Sal	10	18	(6–128)	18	(8–153)
0.1 ng + AVP	22	63	(38–135)‡	54	(17–152)§
0.3 ng + Sal	10	31	(9–127)	18	(9–209)
0.3 ng + AVP	14	107	(23–300)	76	(35–198)
1.0 ng + Sal	17	21	(5–300)	12	(4–200)
1.0 ng + AVP	9	12	(4–300)§	8	(2–300)§
AAVP(V$_2$)					
0.1 ng + Sal	10	16	(13–27)	17	(10–28)
0.1 ng + AVP	21	79	(47–300)¶	40	(18–228)‖
0.3 ng + Sal	10	20	(14–112)	28	(11–55)
0.3 ng + AVP	14	59	(19–247)‡	58	(28–231)‡
1.0 ng + Sal	14	20	(2–300)	15	(3–300)
1.0 ng + AVP	10	8	(2–300)**	8	(2–300)**
AOXT					
0.1 ng + Sal	8	28	(5–76)	28	(4–59)
0.1 ng + AVP	20	115	(21–280)‖	60	(19–206)
0.3 ng + Sal	10	72	(9–105)	32	(27–45)
0.3 ng + AVP	14	45	(32–95)§	28	(19–126)§
1.0 ng + Sal	20	19	(4–222)	18	(3–225)
1.0 ng + AVP	10	21	(2–152)**	22	(4–143)**

n, Number of rats; Sal, saline.
*Median avoidance latencies (in parentheses the 25th–75th percentiles).
†$P < 0.002$ AVP vs. Sal; ‡$P < 0.05$ antagonist + AVP vs. Sal + AVP; §$P < 0.02$ antagonist + AVP vs. Sal + AVP; ¶$P < 0.002$ antagonist + AVP vs. antagonist + Sal; ‖$P < 0.05$ antagonist + AVP vs. antagonist + Sal; **$P < 0.002$ antagonist + AVP vs. Sal + AVP.

To determine the attenuating effect of OXT-like neuropeptides, a higher shock intensity was used. Thus, avoidance latencies were much higher than in previous experiments. Table 3 summarizes the effect of OXT and [pGlu^4,Cyt^6]OXT-(4–8) on passive avoidance behavior. Both 1 and 3 ng significantly inhibited passive avoidance behavior. AOXT, which in itself had a slight, but not significant, attenuating effect on passive avoidance behavior, blocked the effect of 1 and 3 ng of OXT. [pGlu^4,Cyt^6]OXT-(4–8) significantly re-

Table 1. *In vivo* potencies and relative binding affinities of various neurohypophyseal receptor antagonists

Antagonist	Receptor (ref.)	Bioassay*, pA$_2$ *in vivo*: V$_1$	V$_2$	OXT	Relative binding affinity†, pK$_i$: V$_1$	V$_2$	OXT
d(CH$_2$)$_5$[Tyr(Me)^2]AVP	AAVP-V$_1$ (8, 16–18)	8.62	0.31	8.13 (*in vitro*)	9.00	6.66	7.07
Des-Gly,(NH$_2$)^9-d(CH$_2$)$_5$[Tyr(Me^2, Thr^4,Orn^8]vasotocin	OXT (8, 19)	6.48	5.3	7.69	6.28		8.49
d(CH$_2$)$_5$[D-Ile^2,Ile^4]AVP	AAVP-V$_2$ (20–22)	6.42	8.04	6.90			

*Antagonistic potencies are expressed in pA$_2$ values. pA$_2$ is the negative log of A$_2$, which is the concentration of antagonist needed to reduce the response to two times the amount of agonist to equal the response to one time the amount administered before the antagonist.
†Relative binding affinities are given in pK$_i$ values. pK$_i$ is the negative log of the inhibition constant calculated from the IC$_{50}$ value (23).

Table 3. Interaction of OXT-(1–9) with AOXT on retention of passive-avoidance behavior

Treatment	*n*	Passive avoidance behavior*, sec			
		24-hr retention		48-hr retention	
Sal					
+ Sal	10	300	(161–300)	300	(58–300)
+ OXT (1 ng)	11	92	(29–237)†	76	(31–235)
+ OXT (3 ng)	9	17	(11–46)‡	16	(9–125)‡
AOXT					
1 ng + Sal	9	180	(24–300)	75	(7–300)
1 ng + OXT (1 ng)	9	151	(30–300)	92	(32–300)
1 ng + OXT (3 ng)	10	101	(9–173)	50	(27–110)
Sal					
+ Sal	18	176	(73–300)	194	(19–300)
+ OXT-(4–8) (3 pg)	18	47	(9–222)§	38	(7–300)†
+ OXT-(4–8) (10 pg)	18	30	(17–300)§	46	(18–287)
AOXT					
0.3 ng + Sal	6	148	(83–300)	107	(88–273)
0.3 ng + OXT-(4–8) (3 pg)	8	117	(7–300)	228	(23–300)
0.3 ng + OXT-(4–8) (10 pg)	8	20	(10–36)	28	(9–300)
0.3 ng + OXT-(4–8) (30 pg)	9	19	(10–29)¶	16	(12–37)¶
1 ng + OXT-(4–8) (3 pg)	7	300	(7–300)	203	(10–300)
1 ng + OXT-(4–8) (10 pg)	8	258	(31–300)‖	116	(28–300)
1 ng + OXT-(4–8) (30 pg)	8	64	(24–300)	75	(16–146)

n, Number of rats.
*Median avoidance latencies (in parentheses 25th–75th percentiles). †$P < 0.05$; ‡$P < 0.002$; §$P < 0.02$ Sal + OXT or + OXT-(4–8) vs. Sal + Sal; ¶$P < 0.02$ antagonist + OXT-(4–8) vs. antagonist + Sal; ‖$P < 0.05$ antagonist + OXT-(4–8) vs. Sal + OXT-(4–8).

duced passive avoidance behavior in doses of 3 and 10 pg. This effect was blocked by the 1-ng dose but only partly by the 0.3-ng dose of the antagonist.

In accordance with previous data [pGlu^4,Cyt^6]AVP-(4–8) is more potent in facilitating passive avoidance behavior than AVP itself (12). Accordingly [pGlu^4,Cyt^6]AVP-(4–8) already facilitated passive avoidance behavior in a dose of 10 pg (Table 4). Higher doses of this peptide (10 and 30 pg) further facilitated avoidance response. AAVP(V_1) blocked the effect of the smallest dose of [pGlu^4,Cyt^6]AVP-(4–8) (3 pg) but was unable to antagonize the effect of 10- and 30-pg doses. AAVP(V_2) prevented the effect of [pGlu^4,Cyt^6]AVP-(4–8),

Table 4. Interaction of graded doses of [pGlu^4,Cyt^6]AVP-(4–8) with neurohypophyseal receptor antagonists on retention of passive avoidance behavior

Treatment	*n*	Passive avoidance behavior*, sec			
		24-hr retention		48-hr retention	
Sal					
+ Sal	10	27	(8–99)	25	(8–91)
+ AVP-(4–8) (1 pg)	9	47	(14–300)	26	(8–153)
+ AVP-(4–8) (3 pg)	9	138	(82–300)	97	(14–159)
+ AVP-(4–8) (10 pg)	11	145	(70–300)†	172	(81–226)†
+ AVP-(4–8) (30 pg)	9	233	(144–300)‡	251	(132–300)‡
AAVP(V_1)					
+ AVP-(4–8) (3 pg)	9	4	(3–21)§	7	(3–14)§
+ AVP-(4–8) (10 pg)	11	208	(16–300)	42	(11–300)
+ AVP-(4–8) (30 pg)	8	96	(70–240)	88	(45–188)
AAVP(V_2)					
+ AVP-(4–8) (3 pg)	13	86	(35–276)	77	(23–109)
+ AVP-(4–8) (10 pg)	8	57	(17–83)¶	25	(12–90)¶
+ AVP-(4–8) (30 pg)	11	16	(4–60)‖	6	(4–75)¶

n, Number of rats.
*Median avoidance latencies (in parentheses 25th–75th percentiles). †$P < 0.02$; ‡$P < 0.002$ + AVP-(4–8) vs. + Sal; §$P < 0.002$; ¶$P < 0.02$; ‖$P < 0.02$ antagonist + AVP-(4–8) vs. Sal + AVP-(4–8).

Table 5. Interaction of graded doses of [pGlu^4,Cyt^6]AVP-(4–8) with graded doses of AOXT on retention of passive avoidance behavior

Treatment	*n*	Passive avoidance behavior*, sec			
		24-hr retention		48-hr retention	
Sal					
+ Sal	13	13	(9–22)	19	(5–29)
AOXT					
0.1 ng + Sal	8	28	(5–76)	28	(4–59)
0.3 ng + Sal	7	25	(17–74)	18	(6–80)
0.1 ng + AVP-(4–8) (3 pg)	7	38	(21–83)	26	(22–47)
0.1 ng + AVP-(4–8) (10 pg)	7	57	(37–110)	24	(10–76)
0.1 ng + AVP-(4–8) (30 pg)	7	123	(41–243)†	76	(40–164)‡
0.3 ng + AVP-(4–8) (3 pg)	9	8	(6–35)	9	(7–55.5)
0.3 ng + AVP-(4–8) (10 pg)	9	19	(15–93)	14	(10–87)
0.3 ng + AVP-(4–8) (30 pg)	9	88	(18–271)	45	(10–145)
1 ng + AVP-(4–8) (3 pg)	10	32	(12–161)§	24	(10–80)¶
1 ng + AVP-(4–8) (10 pg)	6	15	(10–49)‖	7	(5–14)‖
1 ng + AVP-(4–8) (30 pg)	7	4	(4–45)‖	6	(4–19)§

n, Number of rats.
*Median avoidance latencies (in parentheses 25th–75th percentiles). †$P < 0.05$; ‡$P < 0.02$ antagonist + AVP(4–8) vs. antagonist + Sal; §$P < 0.02$; ¶$P < 0.05$; ‖$P < 0.002$ antagonist + AVP-(4–8) vs. Sal + AVP-(4–8).

although the lowest dose of the neuropeptide was not significantly reduced (Table 5).

AOXT at 1 ng per rat significantly blocked the effect of [pGlu^4,Cyt^6]AVP-(4–8) in all three doses investigated (Table 5). AOXT therefore appeared more potent than the V_1 and V_2 antagonists in blocking the effect of the AVP fragment. A lower dose of AOXT (0.3 ng) significantly blocked the effect of the 3- and 10-pg doses of the fragment, but the lowest dose of 0.1 ng was less effective (Table 6).

Subsequently the influence of the three antagonists was measured on the effect of OXT. Again, the higher shock intensity of 0.5 mA for 2 sec was used. Because 1 ng of the antagonists markedly affected passive avoidance behavior (data not shown), the 0.3-ng dose was used, which also had a blocking effect on AVP-induced facilitation of passive avoidance behavior (see Table 3). Table 6 summarizes the results. OXT at 1 ng significantly attenuated passive avoidance responses. This attenuation was significantly blocked by 0.3 ng of AAVP(V_1) and the same amount of AOXT but

Table 6. Interaction of 1 ng of OXT with 0.3 ng of the various neurohypophyseal receptor antagonists on retention of passive avoidance behavior

Treatment	*n*	Passive avoidance latency*, sec			
		24-hr retention		48-hr retention	
Sal					
+ Sal	10	261	(199–300)	189	(130–299)
+ OXT	14	62	(30–189)†	64	(30–129)
AAVP(V_1)					
+ Sal	14	165	(51–299)	120	(46–300)
+ OXT	12	284	(53–300)‡	138	(107–282)
AAVP(V_2)					
+ Sal	13	300	(179–300)	265	(132–300)
+ OXT	14	61	(36–198)§	44	(15–146)¶
AOXT					
+ Sal	10	150	(105–300)	117	(91–245)
+ OXT	11	275	(154–300)	190	(99–300)

n, Number of rats.
*Median avoidance latencies (in parentheses the 25th–75th percentiles). †$P < 0.02$ OXT vs. Sal; ‡$P < 0.05$ antagonist + OXT vs. Sal + OXT; §$P < 0.02$; ¶$P < 0.05$ antagonist + OXT vs. antagonist + Sal.

not by AAVP(V_2). Both AAVP(V_1) and AOXT as such reduced passive avoidance responses, although not significantly.

In a final series of experiments, the influence of the ancient neurohypophyseal hormone vasotocin ([Arg^8]OXT) was studied. This neuropeptide in "high" doses has a vasopressin-like and in "low" doses has an OXT-like effect on passive avoidance behavior as shown in Fig. 1. The effect of 10 ng of vasotocin was significantly blocked by AOXT at 1 ng, whereas all three antagonists significantly blocked the effect of this neuropeptide at 3 ng (Table 7). To study the attenuating effect of vasotocin on passive avoidance behavior a dose of 0.1 ng was used, and the influence of 0.3 ng of the three antagonists was determined as in the study with OXT (Table 8). Only the AAVP(V_1) and AOXT blocked the effect of vasotocin. Vasotocin-induced attenuation of passive avoidance behavior was not significantly affected by pretreatment with AAVP(V_2).

Table 7. Interaction of 10 ng of vasotocin with various doses of neurohypophyseal receptor antagonists on retention of passive avoidance behavior

Treatment	*n*	Passive avoidance latency*, sec	
		24-hr retention	48-hr retention
Sal			
+ Sal	8	17 (8–33)	15 (13–44)
+ Vtn	14	191 (132–300)‡	114 (90–300)‡
AAVP(V_1)			
1 ng + Vtn	10	97 (22–218)	85 (9–161)
3 ng + Vtn	5	16 (4–27)§	13 (8–20)¶
AAVP(V_2)			
1 ng + Vtn	9	78 (6–296)	78 (5–265)
3 ng + Vtn	6	15 (11–203)¶	12 (4–196)¶
AOXT			
1 ng + Vtn	10	29 (11–245)¶	34 (6–213)¶
3 ng + Vtn	7	4 (4–5)§	5 (4–7)§

n, Number of rats. Vtn, vasotocin.
*Median avoidance latencies (in parentheses the 25th–75th percentiles).
‡$P < 0.002$ Vtn vs. Sal; §$P < 0.02$; ¶$P < 0.05$ antagonist + Vtn vs. Sal + Vtn.

DISCUSSION

These results confirm our previous findings (11) that i.c.v.-administered AVP and related neurohypophyseal neuropeptides—e.g., AVP and [pGlu^4,Cyt^6]AVP-(4–8)—facilitate retention of passive avoidance behavior upon postlearning treatment. In accordance with previous observations (9–11) AAVP(V_1) blocked the behavioral effect of AVP. It dose-dependently inhibited the effect of [pGlu^4,Cyt^6]AVP-(4–8) on passive avoidance behavior. The major finding of these observations is that the interaction of AVP with AAVP(V_1) is not selective. This conclusion is based on the observation that AVP-induced facilitation of passive avoidance behavior was at least as effectively antagonized by AAVP(V_2) and AOXT; the same effect occurred with the AVP fragment [pGlu^4,Cyt^6]AVP-(4–8). The ancient precursor of the neurohypophyseal hormones, vasotocin, has a bimodal effect. In high doses it mimics vasopressin, and in low doses it mimics OXT. The effect of the high as well as the low dose was blocked by AAVP(V_1) and AOXT; AAVP(V_2) was less effective.

The use of antagonists for experiments such as those described in this paper may be questioned when one considers the specificity of these compounds from the data listed in Table 1. This seriously handicaps determination of the receptor involved. We tried to solve this problem by using graded doses of the three antagonists to titrate their relative potency. From these studies we conclude that the three antagonists were almost equally effective in blocking the effect of vasopressin. The attenuating effect of OXT was less well blocked by AAVP(V_2).

This lack of discrimination is not necessarily found with other central nervous system effects of vasopressin. For example, vasopressin-induced hypothermia can be blocked by a dose of 10 ng of AAVP(V_1), whereas 10 times as much is needed when AAVP(V_2) or AOXT is used (D.d.W., unpublished work). Another example is the recent finding (G. Croiset and D.d.W., unpublished work) that pilocarpine-induced epilepsia is attenuated exclusively by a V_2 antagonist. The influence of the neurohypophyseal hormones on avoidance behavior apparently is exerted at a receptor that has the same affinity for AAVP(V_1) and AOXT and a somewhat lesser affinity for AAVP(V_2). Such a receptor might be present in the ventral hippocampus. Here we hypothesize that the receptor implicated in these behavioral effects could well be the so-called OXT receptor.

Binding data have shown that AVP can also bind to OXT receptors (6, 8). The locus of action of neurohypophyseal hormones and related neuropeptides is in the septal hippocampal area—in particular, the ventral subiculum (18). This region mainly contains OXT-binding sites (8). Therefore, the exclusive involvement of V_1 vasopressinergic receptors in mediating the behavioral effects of vasopressin can be questioned.

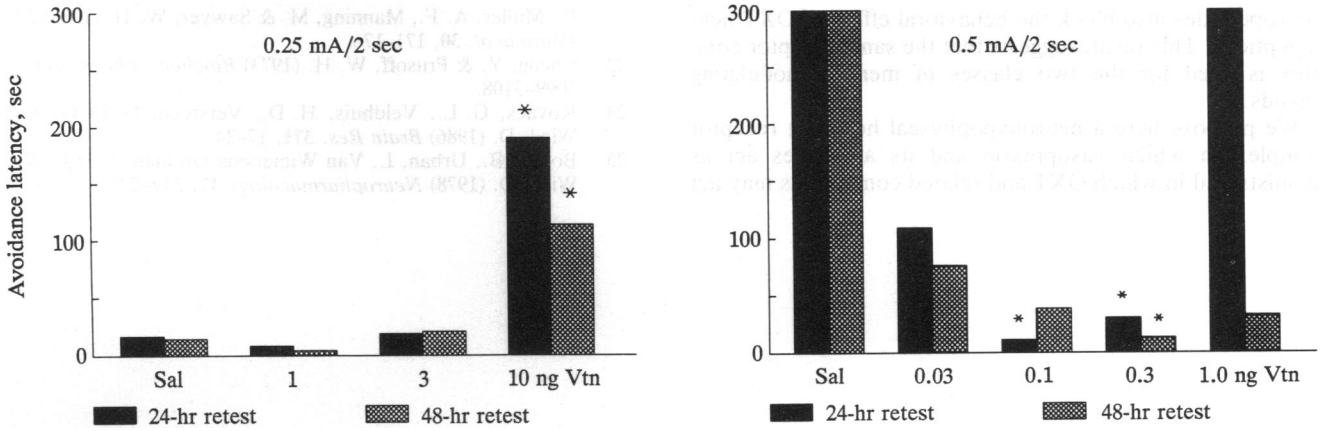

FIG. 1. Effect of high (*Left*) and low (*Right*) doses of vasotocin (Vtn) administered i.c.v. immediately after the learning trial on passive avoidance behavior as determined 24 and 48 hr after the learning trial. To demonstrate facilitation of passive avoidance behavior, rats were exposed to 0.25 mA for 2 sec. For attenuating effects a shock intensity of 0.5 mA for 2 sec was used. (*Left*) *, $P < 0.02$; (*Right*) *, $P < 0.002$.

Table 8. Interaction of 0.1 ng of vasotocin with 0.3 ng of the various neurohypophyseal receptor antagonists on retention of passive avoidance behavior

Treatment	*n*	Passive avoidance latency*, sec			
		24-hr retention		48-hr retention	
Sal					
+ Sal	9	264	(193–300)	244	(166–300)
+ Vtn	12	77	(43–212)†	50	(16–206)‡
AAVP(V_1)					
+ Sal	6	207	(109–300)	104	(64–128)
+ Vtn	12	226	(114–300)	148	(64–300)
AAVP(V_2)					
+ Sal	6	184	(37–300)	87	(41–126)‡
+ Vtn	9	100	(82–285)	73	(22–162)
AOXT					
+ Sal	6	117	(100–300)	111	(88–171)
+ Vtn	11	300	(179–300)§	300	(133–300)

n, Number of rats. Vtn, vasotocin.
*Median avoidance latencies (in parentheses the 25th–75th percentiles).
†$P < 0.02$; ‡$P < 0.05$ Sal + Vtn vs. Sal + Sal; §$P < 0.05$ AOXT + Vtn vs. Sal + Vtn.

Earlier studies showed that the ventral hippocampus, the amygdala, and the septal area were the most effective regions for the effect of neuropeptides related to the neurohypophyseal hormones on passive avoidance behavior (24). These regions and especially the ventral hippocampus are known to express both OXT and AVP receptors. Furthermore, OXT, but also vasotocin and AVP, have equally high affinities for this so-called OXT receptor, whereas OXT has a lower affinity for the peripheral and central AVP receptors. Moreover, the OXT analogue that appears to be a powerful antagonist of the neurohypophyseal peptides on the passive avoidance behavior test has a very low affinity for the AVP receptor and a low antagonistic potency in the vasopressor and antidiuretic assay, whereas it has a high affinity for the hippocampal OXT receptor and a high antagonistic potency in the uterus-contraction assay. Binding studies with brain homogenates indicated that AOXT is very selective for OXT receptors, both in brain and in peripheral tissue. OXT and related peptides have effects opposite those of vasopressin and related peptides (25). These amnesic peptides attenuate active and passive avoidance behavior. Indeed, administered i.c.v., OXT also dose-dependently attenuated passive avoidance behavior in the present experiments. This effect was readily blocked by AOXT. AAVP(V_1) but not AAVP(V_2) also blocked the behavioral effect of OXT. Thus, the same antagonists that block the behavioral effect of vasopressin neuropeptides also block the behavioral effect of OXT neuropeptides. This result suggests that the same receptor complex is used for the two classes of memory-modulating ligands.

We propose here a neurohypophyseal hormone receptor complex in which vasopressin and its analogues act as agonists and in which OXT and related compounds may act as "inverse" agonists. Both the agonistic and "inverse" agonistic actions can be blocked by AOXT. This concept seems useful as a functional model. However, no direct molecular proof exists for such a putative receptor complex. The model may provide an explanation for blocking of the vasopressin fragment [pGlu^4,Cyt^6]AVP-(4–8) and the OXT fragment [pGlu^4,Cyt^6]OXT-(4–8) effect by AOXT.

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