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Vasopressin Antagonists Block Peripheral as Well as Central Vasopressin Receptors[1]

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DE WIED, D., O. GAFFORI, J. M. VAN REE AND W. DE JONG. *Vasopressin antagonists block peripheral as well as central vasopressin receptors.* PHARMACOL BIOCHEM BEHAV 21(3) 393–400, 1984.—The aim of the present study was to differentiate between the postulated central behavioral effects of vasopressin and its pressor response, which is mainly mediated by peripheral vascular receptors. Thus, the interaction between the vasopressor antagonists dPTyr(Me)AVP (AAVP[a]) and d(CH_2)$_5$Tyr(Me)AVP (AAVP[b]) with the effects of [Arg[8]]vasopressin (AVP-(1–9)) and [pGlu[4],Cyt[6]]AVP-(4–8) (referred to as AVP-(4–8)) was examined using passive avoidance behavior and the pressor response as parameters. AVP-(4–8) was approximately 4 and 200 times more potent than AVP-(1–9) in facilitating passive avoidance behavior after subcutaneous (SC) or intracerebroventricular (ICV) administration respectively. This effect of SC injected AVP-(1–9) and AVP-(4–8) could be prevented by both vasopressor antagonists following SC treatment. A similar antagonistic action was found when AVP-(1–9) or AVP-(4–8) and the antagonist AAVP[b] were administered ICV. SC injection of AAVP[b] prevented the behavioral effect of ICV administered AVP-(1–9) while ICV treatment with the antagonist blocked the behavioral action of systemically injected AVP-(1–9) and AVP-(4–8). In contrast to SC injected AVP-(1–9) which dose-dependently increased blood pressure and decreased heart rate, AVP-(4–8) injected SC in identical doses did not affect blood pressure and heart rate, neither did AVP-(1–9) and AVP-(4–8) when injected ICV in behaviorally active doses. A SC, but not an ICV injection of the antagonist AAVP[b] could prevent the blood pressure increase and bradycardia induced by SC AVP-(1–9). The present data indicate that the receptors involved in the action of vasopressin on passive avoidance behavior are clearly separated from those involved in the pressor response with respect to both location and structural requirement for activation. The receptors mediating the behavioral effect are likely present in the central nervous system, whereas those involved in the pressor response are mainly located in the periphery. AVP-(4–8) potently mimics AVP-(1–9) in facilitating passive avoidance behavior, without possessing a direct pressor effect. These findings support the postulate that the behavioral effects of vasopressin are mediated by neuropeptides generated from this nonapeptide in the brain synaptic membranes [8] and due to a direct action on the brain.

Vasopressin Vasopressin fragments Vasopressor antagonists Passive avoidance behavior Blood pressure response

THE neurohypophyseal hormone vasopressin which exerts antidiuretic and vasoconstrictor effects may also function as a modulator of memory processes [9,11]. It increases resistance to extinction of active avoidance behavior, facilitates passive avoidance behavior and reverses or prevents experimental amnesia [1, 5, 9, 13, 21, 22]. Data on passive avoidance behavior suggest that vasopressin enhances both consolidation and retrieval of recently acquired information. These effects can be elicited following intracranial administration in much lower doses than following systemic administration, suggesting a central action [10]. Accordingly, during a passive avoidance task the concentration of immunoreactive vasopressin was markedly decreased in various brain structures [17]. Studies on the site of action showed that limbic midbrain structures are involved in the behavioral response to vasopressin [15,23].

The behavioral influence of vasopressin is dissociated from its classical peripheral endocrine effects since fragments of vasopressin having little or no antidiuretic and blood pressure increasing effects retain the influences on avoidance behavior [10,12]. Burbach *et al.* [8] have shown that vasopressin can be converted to fragments by rat brain synaptic membranes which act markedly stronger than the parent hormone. The des-glycinamide derivative of one of these vasopressin fragments is the peptide [pGlu[4],Cyt[6]] AVP-(4–8) which enhances passive avoidance latency in much lower amounts than compared to those of vasopressin. This fragment appears to be devoid of pressor activity.

Le Moal *et al.* [18] and Koob *et al.* [14] have shown that the vasopressin antagonist dPTyr(Me)AVP which prevents the pressor response of vasopressin [3] also abolishes the influence of this hormone on extinction of active avoidance behavior. These authors suggest that this blockade indicates that signals from peripheral, physiological and endocrine sources may have an important role in the behavioral effect of vasopressin. The present experiments were designed to

[1]Dedicated to Professor Dr. E. J. Ariëns, Department of Pharmacology, Medical Faculty, University of Nijmegen, on the occasion of his retirement.

evaluate this hypothesis, using passive instead of active avoidance behavior as the behavioral test procedure. The vasopressin antagonists dPTyr(Me)AVP and the more potent $d(CH_2)_5$Tyr(Me)AVP were used and their blocking action on the influence of [Arg^8]vasopressin (AVP-(1–9)) and [pGlu^4,Cyt^6]-AVP-(4–8) on blood pressure and passive avoidance behavior was determined.

The results indicate that the vasopressin antagonists block peripheral as well as central receptors for vasopressin and that the memory effect of vasopressin can be dissociated from its peripheral pressor effect.

METHOD

Animals

Male Wistar rats of an inbred strain (CPB-TNO, Zeist, The Netherlands) weighing 140–160 g, were used. The animals were housed 5 per cage at room temperature (20–21°C). All animals had access to commercial food and tap water ad lib and were kept on a controlled illumination schedule (light on between 6 a.m. and 8 p.m.). They were handled on two consecutive days prior to the start of the experiment. Rats were transported from the animal house to the experimental room 1 hr before the experiment. All observations were made between 1 p.m. and 6 p.m.

Intracerebroventricular Cannulation

For intracerebroventricular (ICV) injection rats were equipped with a polyethylene cannula in the lateral ventricle. The operation was performed under ether anesthesia and aseptic conditions. A hole was drilled through the skull, 1 mm lateral to the midline and 0.5 mm caudal to the bregma for insertion of the cannula. After insertion, the cannula was fixed to the skull by means of dental cement on 2 stainless steel screws. After the operation, the rats were housed in separate cages and allowed to recover from the operation for at least one week.

Passive Avoidance Behavior

Animals were trained in a step-through type one trial learning passive avoidance test [2]. The experimental apparatus consisted of an illuminated platform (40 W bulb, placed 30 cm above) attached to a large, dark compartment equipped with a grid floor. After habituation to the dark compartment (2 min), rats were placed on the platform and allowed to enter the dark compartment; since rats prefer dark to light, they normally entered within 15 sec. On the next day after three more trials, an unavoidable scrambled footshock (0.25 mA, 2 sec) was delivered through the grid floor of the dark compartment (learning trial). The median entrance latencies at the learning trial for the different groups in the various experiments, ranged from 3 to 10 sec, and the differences between groups were not significant. Rats were removed from the shock box 10 sec after the termination of the shock. Great care was taken to ensure that animals actually received the shock: (1) the current level was frequently verified by direct measurement; (2) the shock delivery was regularly monitored by the experimenter (fingers on the grid); (3) behavior of every animal was observed; when they received the shock, animals displayed obvious signs of mild distress (running, jumping, squeaking); (4) metal grids and walls were scrupulously cleaned with dry towels between experiments and between the learning trials of the animals. Passive avoidance latencies were tested 24 hr and 48 hr after the learning trial; the rats were placed on the platform and the latency to enter the dark compartment was measured up to a maximum of 300 sec. The subjects were tested in a predetermined order to diminish bias in the results. In order to assess the influence of peptides on the storage of the aversive experience (memory consolidation), peptide or placebo were injected immediately after the learning trial. In the case that two injections were administered, the first injection (antagonist) was given directly after the learning trial and the second 30 minutes later (agonist).

Blood Pressure

Rats (200–220 g) received an indwelling cannula (PE 50) in the ventral tail artery under ether anesthesia. Blood pressure registration commenced 20–24 hr later using a Statham transducer (P 23 Ac) and a Grass polygraph (model 7 D) while the rats were in their home cage. After stabilization of blood pressure and heart rate subcutaneous (SC) or ICV injections were given and the effects were assessed for 1–2 hr.

Peptides and Injection Procedures

[Arg^8]vasopressin (AVP-(1–9)), [pGlu^4,Cyt^6]-AVP-(4–8) (in this paper referred to as AVP-(4–8)) and two AVP vasopressor antagonists were used. The first antagonist dPTyr(Me)AVP (AAVP^a) has an antivasopressor pA_2 of 7.96 [3]. The second, more potent, antagonist $d(CH_2)_5$Tyr(Me)AVP (AAVP^b) has an antivasopressor pA_2 of 8.62 [16]. The peptides were dissolved in one drop of 10^{-5} N HCl and then diluted with saline (0.9% NaCl, pH 6.5–6.7). Subcutaneous injections were given in a volume of 0.5 ml/rat. For intraventricular injections 1 μl/rat was injected using a Hamilton syringe. A needle (0.25 mm outer diameter) adapted to the appropriate length was inserted into the guide cannula. Control animals received 1 μl saline (placebo).

Histological Control

The localization of the tip of the cannula was determined at the termination of the experiment by the injection of Evans blue. The staining was then inspected macroscopically in formaldehyde fixated brain sections. Data from rats where the dye did not stain the walls of the lateral and the third ventricles were discarded from the analyses.

Statistical Analysis

Passive avoidance latencies were analysed with Kruskal-Wallis testing and subsequently with Mann-Whitney non-parametric tests. ED_{50}'s were calculated following the method of Litchield and Wilcoxon [19]; as response was taken a latency of more than 190 sec (only one of 97 placebo treated rats scored positive). Blood pressure values were analyzed with Student's t-tests.

RESULTS

Passive Avoidance Behavior

In the first series of experiments, the influence of graded doses of the vasopressin agonists and antagonists on passive avoidance behavior was studied following SC injection immediately after the learning trial. Both AVP-(1–9) and AVP-(4–8) induced a dose-dependent facilitation of passive avoidance behavior at the 24 and 48 hr retention test (Table 1). The data of the 24 hr retention test were used to calculate

TABLE 1
EFFECTS OF VASOPRESSIN AND RELATED PEPTIDES ON RETENTION OF PASSIVE AVOIDANCE BEHAVIOR

treatment[1] and dose per rat	N[2]	latency (median sec) first retention test 24 hr[3]		second retention test 48 hr	
AVP-(1–9)					
0.1 μg	6	110[4]	(26–188)	120	(25–187)
0.3 μg	6	155*	(83–300)	115	(21–300)
1 μg	6	179*	(83–300)	210†	(168–300)
3 μg	6	220†	(181–300)	240†	(137–300)
placebo	12	78	(24–145)	69	(18–107)
AVP-(4–8)					
0.01 μg	6	120	(31–189)	107	(26–170)
0.03 μg	6	139*	(98–180)	110	(40–175)
0.1 μg	6	243†	(180–300)	163*	(75–300)
0.3 μg	6	300‡	(267–300)	300‡	(187–300)
placebo	12	78	(24–145)	69	(18–107)
AAVP[a]					
1.5 μg	7	42	(32–48)	35	(16–70)
5 μg	7	16†	(4–36)	34	(14–38)
15 μg	7	56	(18–180)	48	(13–50)
placebo	7	81	(37–300)	61	(28–110)
AAVP[b]					
0.3 μg	6	28*	(4–64)	24*	(7–51)
1 μg	6	17†	(4–37)	17†	(8–41)
3 μg	6	118	(47–200)	107	(38–133)
placebo	6	106	(41–208)	100	(33–183)

Graded doses of the peptides were SC injected immediately after the learning trial.
[1]The peptides tested were: AVP-(1–9): [Arg[8]] vasopressin
AVP-(4–8): [pGlu[4],Cyt[6]]AVP-(4–8)
AAVP[a]: dPTyr(Me)AVP
AAVP[b]: d(CH_2)$_5$Tyr(Me)AVP
[2]Number of animals.
[3]Hr after the learning trial.
[4]Values are given as median with in brackets the 25th and 75th percentile.
Different from rats simultaneously treated with placebo ($p<0.05$; †$p<0.02$; ‡$p<0.002$); Kruskal-Wallis test and subsequently Mann-Whitney U-test).

ED_{50}'s. The ED_{50} for AVP appeared 0.57 μg and that for AVP-(4–8) 0.105 μg. Thus, on a molar base AVP-(4–8) is approximately 4 times more potent than AVP-(1–9) in this test procedure after SC administration. In contrast to the vasopressin agonists, the vasopressin antagonists attenuated passive avoidance behavior (Table 1). The vasopressin antagonist AAVP[b], which is more potent than AAVP[a] with respect to antivasopressor action, was also more effective in attenuating passive avoidance behavior. At higher doses this influence of both antagonists disappeared.

Next, the interaction between the vasopressin agonists and antagonists was investigated following SC treatment. The vasopressin antagonists were administered in relatively high doses, because these doses did not affect passive avoidance behavior per sé (Table 1). Again, a dose related facilitation of passive avoidance behavior was observed after treatment with AVP-(1–9) and AVP-(4–8) (Table 2, Fig. 1). Both vasopressin antagonists partly or completely antagonized the facilitating action of AVP-(1–9) (Table 2). Similar data were obtained when AVP-(4–8) was used as agonist (Fig. 1; results with AAVP[a] are not shown). The effect of two doses (3 and 10 μg) of AAVP[b] was explored in this experiment. As can be inferred from the dose response curves of AVP-(4–8) in the absence and presence of the vasopressin antagonist, the antagonism between these peptides is competitive in nature (Fig. 1). There is a parallel shift of the curves to the right in the presence of the antagonist.

Subsequently, similar experiments were performed following ICV injection. A dose-related facilitation of passive avoidance behavior was observed after injection of AVP-(1–9) and AVP-(4–8) at both the 24 and 48 hr retention test (Table 3). The ED_{50}'s for these peptides, calculated on the data obtained at the 24 hr retention test, were for AVP-(1–9) 1.3 ng and for AVP-(4–8) 4.5 pg. Thus, on a molar base AVP-(4–8) is about 200 times more potent than AVP-(1–9) after ICV administration. Comparing SC and ICV injection of these peptides, it appeared that the peptides are much more potent when administered directly into the brain ven-

TABLE 2

EFFECT OF THE VASOPRESSIN ANTAGONISTS AAVP[a] (dPTyr(Me)AVP) AND AAVP[b] (d(CH_2)$_5$Tyr(Me)AVP) ON THE INFLUENCE OF AVP-(1-9) ([Arg^8]VASOPRESSIN) ON THE RETENTION OF PASSIVE AVOIDANCE BEHAVIOR

treatment and dose per rat	N[1]	latency (sec) first retention test 24 hr[2]		second retention test 48 hr	
placebo + placebo	6	81[3]	(51–180)	65	(47–110)
AAVP[a] 15 μg + placebo	6	65	(28–186)	48	(42–112)
placebo + AVP-(1-9) 0.3 μg	6	150*	(116–300)	130*	(47–240)
placebo + AVP-(1-9) 3 μg	6	246†	(83–300)	181†	(71–300)
AAVP[a] 15 μg + AVP-(1-9) 0.3 μg	6	116	(51–120)	35§	(17–71)
AAVP[a] 15 μg + AVP-(1-9) 3 μg	6	166‡	(48–300)	72‡	(43–106)
placebo + placebo	6	101	(51–162)	81	(31–113)
AAVP[b] 3 μg + placebo	6	94	(42–127)	77	(37–101)
placebo + AVP-(1-9) 0.3 μg	6	193*	(156–300)	128*	(79–221)
placebo + AVP-(1-9) 1 μg	6	221†	(177–232)	180†	(169–240)
placebo + AVP-(1-9) 3 μg	6	267†	(163–300)	221†	(181–253)
AAVP[b] 3 μg + AVP-(1-9) 0.3 μg	6	34§	(13–53)	43‡	(18–60)
AAVP[b] 3 μg + AVP-(1-9) 1 μg	6	52§	(29–79)	49‡	(12–97)
AAVP[b] 3 μg + AVP-(1-9) 3 μg	6	99§	(18–127)	70‡	(13–112)

Peptides were SC injected immediately (antagonists) and 30 min (AVP-(1-9)) after the learning trial.
[1]Number of animals; [2]hr after the learning trial; [3]values are given as median within brackets the 25th and 75th percentile; *different from placebo, placebo treated rats (*$p<0.05$; †$p<0.02$, Kruskal-Wallis test and subsequently Mann-Whitney U-test); ‡different from rats treated with placebo instead of the antagonist (‡$p<0.05$; §$p<0.02$).

tricle (approximately 440 and 23,000 times for AVP-(1-9) and AVP-(4-8) respectively). The vasopressin antagonist AAVP[b] was selected for ICV administration. ICV injection of 3 ng of this peptide which did not influence passive avoidance latencies in placebo treated rats, antagonized the facilitatory action of AVP-(1-9) as well as AVP-(4-8) (Table 3). The dose response curves for the vasopressin agonists in the absence and presence of the vasopressin antagonist again revealed a competitive antagonism.

The vasopressin antagonist AAVP[b] antagonized the behavioral effects of ICV injected AVP-(1-9) when the antagonist was administered into the brain ventricle (Table 3), but also when the antagonist was SC injected (Table 4). Thus, a SC injection of 3 μg AAVP[b] completely prevented the facilitatory effect of an ICV injection of 3 ng AVP-(1-9). The effect of a SC injection of 3 μg of AVP-(1-9) or 0.3 μg of AVP-(4-8) could be prevented by ICV pretreatment with 3 ng of AAVP[b] (Table 4).

Injection of AVP-(1-9) into the lateral ventricle caused barrel rotation in a number of rats. This was never observed after similar treatment with AVP-(4-8) at any of the doses used. Barrel rotation following AVP-(1-9) treatment did not occur in the presence of the antagonist.

Blood Pressure

The effect of different doses of AVP-(1-9) and AVP-(4-8) employed for the passive avoidance test, were determined on blood pressure and heart rate following SC and ICV administration. AVP-(1-9) induced a dose-dependent increase in blood pressure and decrease in heart rate after SC treatment (Fig. 2). Maximal effects were observed between 10 and 30 min after injection. Identical doses of AVP-(4-8) did not significantly affect blood pressure and heart rate (Table 5). For ICV administration the following doses of the peptides were selected: for AVP-(1-9): 0.3, 3.0 and 30 ng; and

TABLE 3

EFFECT OF THE VASOPRESSIN ANTAGONIST AAVP[b] (d(CH_2)$_5$ Tyr(Me)AVP) ON THE INFLUENCE OF AVP-(1-9) ([Arg[8]]VASOPRESSIN) OR AVP-(4-8) ([pGlu[4],Cyt[6]]AVP-(4-8)) ON THE RETENTION OF PASSIVE AVOIDANCE BEHAVIOR

treatment and dose per rat	N[1]	latency (sec) first retention test 24 hr[2]		second retention test 48 hr	
placebo + placebo	18	80[3]	(34–105)	68	(23–81)
AAVP[b] + placebo	18	84	(25–110)	73	(24–103)
placebo + AVP-(1–9) 0.1 ng	6	107	(37–121)	88	(31–121)
placebo + AVP-(1–9) 0.3 ng	6	155*	(122–230)	133*	(120–201)
placebo + AVP-(1–9) 3 ng	6	220†	(138–300)	141*	(130–300)
placebo + AVP-(1–9) 10 ng	6	300‡	(220–300)	201†	(183–300)
placebo + AVP-(1–9) 30 ng	6	300‡	(273–300)	230†	(200–300)
AAVP[b] 3 ng + AVP-(1–9) 0.3 ng	6	103	(41–140)	91	(12–151)
AAVP[b] 3 ng + AVP-(1–9) 3 ng	6	107§	(40–122)	100	(24–113)
AAVP[b] 3 ng + AVP-(1–9) 10 ng	6	220§	(83–300)	147§	(67–300)
AAVP[b] 3 ng + AVP-(1–9) 30 ng	6	280	(160–300)	226	(137–300)
placebo + placebo	18	79	(79–101)	69	(20–83)
AAVP[b] 3 ng + placebo	18	81	(31–120)	71	(20–97)
placebo + AVP-(4–8) 0.1 pg	6	107	(37–112)	92	(34–101)
placebo + AVP-(4–8) 0.3 pg	6	151*	(79–183)	127*	(69–131)
placebo + AVP-(4–8) 3 pg	6	200†	(185–300)	151*	(86–170)
placebo + AVP-(4–8) 30 pg	6	267†	(190–300)	221†	(151–300)
placebo + AVP-(4–8) 100 pg	6	300‡	(221–300)	231†	(201–300)
placebo + AVP-(4–8) 300 pg	6	300‡	(273–300)	230†	(200–300)
AAVP[b] 3 ng + AVP-(4–8) 3 pg	6	88¶	(20–99)	80§	(15–87)
AAVP[b] 3ng + AVP-(4–8) 30 pg	6	72¶	(22–127)	60¶	(17–81)
AAVP[b] 3 ng + AVP-(4–8) 100 pg	6	200§	(89–300)	161§	(61–202)
AAVP[b] 3 ng + AVP-(4–8) 300 pg	6	276	(137–300)	238	(119–300)

Peptides were ICV injected immediately (antagonists) and 30 min (AVP-(1–9) or AVP-(4–8)) after the learning trial.
[1]Number of animals; [2]hr after the learning trial; [3]values are given as median within brackets the 25th and 75th percentile; *different from placebo, placebo treated rats (*$p<0.05$; †$p<0.02$; ‡$p<0.002$, Kruskal-Wallis test and subsequently Mann-Whitney U-test); §different from rats treated with placebo instead of the antagonist (§$p<0.05$; ¶$p<0.02$).

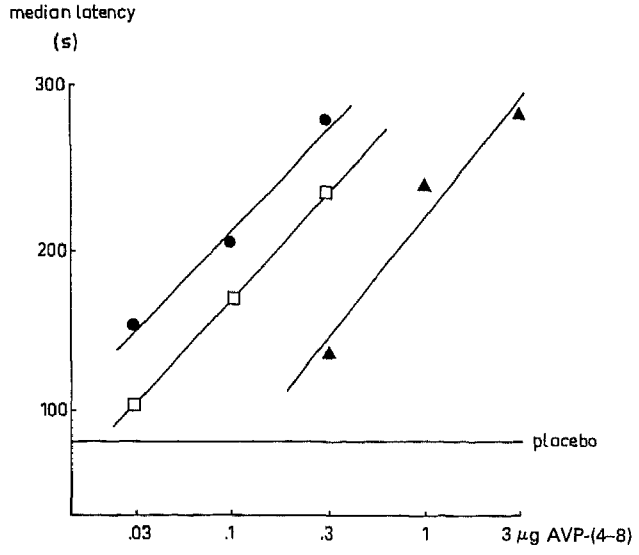

FIG. 1. Antagonism between the vasopressin antagonist AAVP^b (d$(CH_2)_5$ Tyr(Me)AVP and AVP-(4–8) ([pGlu^4,Cyt^8]AVP-(4–8)) following SC treatment using passive avoidance behavior as test parameter. AAVP^b was injected immediately after the learning trial and AVP-(4–8) 30 min later. Retention of the response was measured by timing the latency to enter the dark compartment 24 hr after the learning trial. The dose (μg) of AVP-(4–8) per animal is plotted versus the median of the measured latencies in seconds [2]. ●placebo + AVP-(4–8); □ AAVP^b (3 μg) + AVP-(4–8); ▲ AAVP^b (10 μg) + AVP-(4–8). Lines were calculated with the methods of least squares.

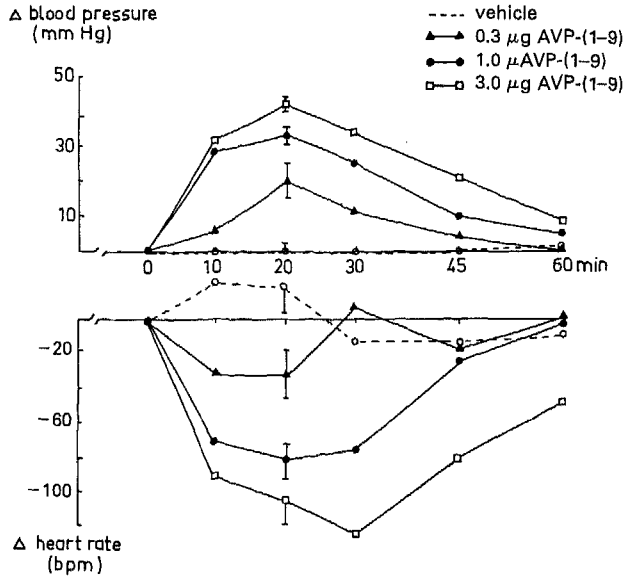

FIG. 2. Effect of SC administration ofAVP-(1–9) on blood pressure and heart rate of conscious rats. Changes in blood pressure (Δ mm Hg) and in heart rate (Δ bpm) are plotted versus the time after injection (min). Data are means of 5 animals. At 20 min SEM is indicated by bars. Basal values of blood pressure and heart rate were respectively 107±4 mm Hg and 403±15 bpm.

for AVP-(4–8): 3, 30 and 300 pg. None of the doses of the peptides had any effect on blood pressure and heart rate (data not shown).

The vasopressin antagonist AAVP^b did not change the blood pressure and heart rate after SC (3 μg, Table 5) or ICV (3 ng) injection. SC treatment with AAVP^b (3 μg) completely prevented the blood pressure increase and bradycardia induced by AVP-(1–9) (Fig. 3 for blood pressure data). In contrast, 3 ng AAVP^b injected into the brain ventricle had no influence on the increase in blood pressure and the bradycardia, induced by SC treatment with AVP-(1–9) (Fig. 3).

DISCUSSION

[Arg^8]vasopressin facilitates passive avoidance behavior when given either immediately after the learning trial or before the retention test [6]. In the present experiments AVP-(1–9) and the fragment AVP-(4–8) ([pGlu^4,Cyt^6]-AVP-(4–8)) were administered immediately after the learning trial. Both peptides facilitated passive avoidance behavior following SC as well as ICV administration. Burbach *et al.* [8] found that the fragment AVP-(4–8) is markedly more potent than the parent molecule. These findings are substantiated and extended by the present data, which support the notion that the behavioral effects of vasopressin are mediated by a direct effect on the central nervous system and are dissociated from the peripheral pressure effect of this hormone [12]. Arguments in favour are the following: Both AVP-(1–9) and AVP-(4–8) were much more potent in facilitating passive avoidance behavior when injected into the cerebral ventricle than following systemic (SC) treatment (440 and 23,000 times respectively). Although systemic (SC) AVP-(4–8) was several times more potent than systemic (SC) AVP-(1–9) on passive avoidance behavior it did not affect blood pressure or heart rate when injected SC in doses in which AVP-(1–9) markedly increased blood pressure and decreased heart rate. Intracranial amounts of AVP-(1–9) and AVP-(4–8) that facilitated passive avoidance behavior did not affect blood pressure or heart rate. ICV pretreatment with the vasopressin antagonist AAVP^b, which did not affect AVP-(1–9)-induced blood pressure increase and heart rate decrease following systemic (SC) injection, antagonized the behavioral effect of ICV injected AVP-(1–9). ICV injected AAVP^b antagonized the effect of systemically (SC) administered AVP-(1–9) and AVP-(4–8) on passive avoidance behavior, while ICV injection of AVP-(1–9) did not change blood pressure and heart rate. Although the present data confirm the findings of Le Moal *et al.* [18] and Koob *et al.* [14], which show that vasopressor antagonists can block the behavioral effects of vasopressin, we disagree with the conclusion of these authors that this is partly the result of blocking the peripheral effects of vasopressin. The present data indicate that systemically injected vasopressin antagonists can penetrate the brain in sufficient amounts to prevent the memory effect of AVP-(1–9) and can block putative vasopressin receptors in the brain. Consistently, it was recently found that the vasopressin antagonists used in the present experiments compete with AVP-(1–9) for binding to vasopressin binding sites in the brain [4]. Evidence has been presented that brain receptors for oxytocin can be antagonized by a struc-

TABLE 4

EFFECT OF THE VASOPRESSIN ANTAGONIST AAVP[b] (d(CH$_2$)$_5$Tyr(Me)AVP) ON THE INFLUENCE OF AVP-(1–9) ([Arg^8]VASOPRESSIN) OR AVP-(4–8) ([pGlu^4,Cyt^6]AVP-(4–8)) ON THE RETENTION OF PASSIVE AVOIDANCE BEHAVIOR

treatment and dose per rat		N[1]	latency (sec) first retention test 24 hr[2]		second retention test 48 hr	
SC (0 min)	ICV (30 min)					
placebo	placebo	6	77 [3]	(44–112)	61	(23–78)
AAVP[b] 3 μg	placebo	6	81	(21–110)	70	(18–100)
placebo	AVP-(1–9) 3 ng	6	280†	(264–300)	165*	(62–300)
AAVP[b] 3 μg	AVP-(1–9) 3 ng	6	57§	(11–69)	83‡	(50–155)
ICV (0 min)	SC (30 min)					
placebo	placebo	6	82	(56–93)	62	(29–70)
AAVP[b] 3 ng	placebo	6	95	(82–131)	82	(68–121)
placebo	AVP-(1–9) 3 μg	6	300†	(183–300)	300	(162–300)
AAVP[b] 3 ng	AVP-(1–9) 3 μg	6	69§	(54–103)	65	(47–88)
ICV (0 min)	SC (30 min)					
placebo	placebo	6	77	(44–112)	61	(23–78)
AAVP[b] 3 ng	placebo	6	70	(13–110)	63	(10–101)
placebo	AVP-(4–8) 0.3 μg	6	275†	(176–300)	140*	(64–189)
AAVP[b] 3 ng	AVP-(4–8) 0.3 μg	6	99§	(22–172)	81‡	(17–105)

Peptides were SC or ICV injected immediately (0 min) and 30 min after the learning trial.
[1]Number of animals; [2]hr after the learning trial; [3]values are given as median within brackets the 25th and 75th percentile; *different from placebo, placebo treated rats (*$p<0.05$; †$p<0.02$, Kruskal-Wallis test and subsequently Mann-Whitney U-test); ‡different from treated with placebo instead of the antagonist (‡$p<0.05$; §$p<0.02$).

TABLE 5

EFFECT OF S.C. ADMINISTRATION OF [Arg^8]VASOPRESSIN (AVP-(1–9)), [pGlu^4,Cyt^6]AVP-(4–8) (AVP-(4–8)) AND d(CH$_2$)$_5$Tyr(Me)AVP (AAVP[b]) ON BLOOD PRESSURE AND HEART RATE

treatment and dose per rat	N[1]	blood pressure[2] (Δ mmHg)	heart rate[2] (Δ bpm)
placebo	6	3 ± 2	−18 ± 12
3.0 μg AVP-(1–9)	6	47 ± 5*	−102 ± 6*
0.3 μg AVP-(4–8)	6	0 ± 1	−12 ± 10
1.0 μg AVP-(4–8)	6	−1 ± 2	−14 ± 13
3.0 μg AVP-(4–8)	6	0 ± 2	−3 ± 6
3.0 μg AAVP[b]	6	5 ± 3	−6 ± 17

[1]Number of animals.
[2]Data are expressed as mean (±SEM) changes in blood pressure (Δ mmHg) and heart rate (Δ bpm) at 15 min after injection as compared to baseline values, obtained before treatment. Baseline values of all rats: blood pressure 111 ± 2 mmHg, heart rate 401 ± 6 bpm. *$p<0.01$.

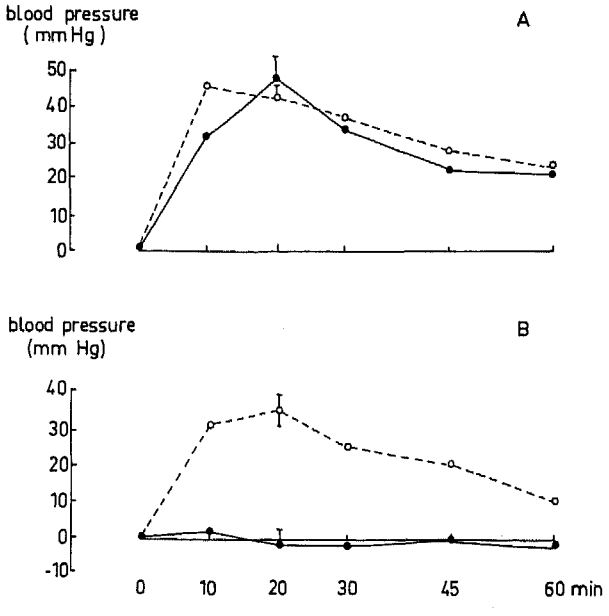

FIG. 3. Interaction between ICV (A) or SC (B) administered AAVP[b] (d(CH$_2$)$_5$Tyr(Me)AVP) and SC injected AVP-(1–9) ([Arg^8]vasopressin) using the blood pressure as test parameter. AAVP[b] (●—●) at a dose of 3 ng (ICV) and 3 μg (SC) or placebo (○—○) was injected 30 min before the administration of 3 μg AVP-(1–9). Changes in blood pressure (Δ mm Hg) are plotted versus the time after the last injection (min). Data are means of 4–5 animals. At 20 min SEM is indicated by bars. Basal values of all rats were 106±2 mm Hg.

tural analogue known to block the action of oxytocin [20]. Whether these brain receptors can also be activated by behaviorally potent fragments of oxytocin accumulating during the incubation of oxytocin with brain synaptic membranes [7], remains however to be shown.

The vasopressin receptors involved in the action of vasopressin on passive avoidance behavior ("behavioral" re-

ceptor) are clearly separated from those concerned in the pressor response ("pressor" receptor) with respect to both location and structural requirements for activation. As argued before, the "behavioral" receptors are likely present in the central nervous system, while the "pressor" receptors are mainly located in the periphery. Although both receptor systems seem to be sensitive for the vasopressin antagonists, the peptide AVP-(4–8) activates the behavioral receptor only. The same holds for DGAVP (AVP-(1–8)), in which the behavioral action of vasopressin is retained in contrast to its pressor and other peripheral activities [12].

Koob *et al.* [14] found that the vasopressin antagonist $AAVP^a$ produced a dose-dependent facilitation of extinction of pole-jumping avoidance behavior. In the present experiments using passive avoidance behavior as the test procedure we found that $AAVP^a$ and $AAVP^b$ attenuated this behavior only when low doses were injected. Higher amounts were ineffective in this respect, which may be due to some agonistic activity of these peptides. Indeed, it has been shown that these antagonists exhibit slight antidiuretic activity [3,16]. Thus, the antagonists may be regarded as mixed agonist/antagonists for peripheral but also for central effects.

The peptide [$pGlu^4$,Cyt^6]-AVP-(4–8) (AVP-(4–8)) very potently mimicked the facilitatory action of AVP-(1–9) on passive avoidance behavior. This peptide is derived from [$pGlu^4$,Cyt^6]-AVP-(4–9). This latter peptide can be generated from AVP-(1–9) by brain synaptic membranes [8] and may be present in the brain (unpublished data). This and related peptides may therefore be regarded as important endogenous ligands for modulation of behavioral processes. If this can be substantiated, vasopressin may be regarded as a precursor molecule of endogenous ligands involved in memory processes. Although vasopressin has been important for discovery of the behavioral effects of this hormone, in future studies the more specific fragments which may be generated from vasopressin in the brain such as [$pGlu^4$,Cyt^6]-AVP-(4–8) should be used to further analyze the implications of the central effects of neurohypophyseal hormones.

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