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Effects of Vasopressin on Noradrenaline-Induced Cyclic AMP Accumulation in Rat Brain Slices

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HAMBURGER-BAR, R. AND M. E. NEWMAN. *Effects of vasopressin on noradrenaline-induced cyclic AMP accumulation in rat brain slices.* PHARMACOL BIOCHEM BEHAV 22(2) 183–187, 1985.—Addition of arginine vasopressin (AVP) or 1-desamino-8-D-arginine vasopressin (DDAVP) to rat cortical slices resulted in significant inhibition of the rise in cyclic AMP produced by incubation with 50 μM noradrenaline. A single injection of DDAVP (20 μg/rat) produced a reduced response to noradrenaline in derived cortex and caudate slices. In animals pretreated at day 5 of life with IP desipramine and intracisternal 6-hydroxydopamine (6-OHDA), both acute and chronic treatments with DDAVP resulted in a reduction in response in derived cortical, caudate and hippocampal slices. The 6-OHDA pretreated animals also showed reduced open-field behavioural activity after both acute and chronic DDAVP, while animals which were not pretreated responded to acute treatment only. The relationship between the effects of vasopressin on noradrenaline-induced cyclic AMP accumulation and its action on learning and memory is discussed.

Vasopressin DDAVP Noradrenaline Cyclic AMP Brain area Learning

VASOPRESSIN is present in several areas of the brain and affects animal learning and behaviour [6]. It facilitates active and passive avoidance learning in rats, possibly by interactions with brain catecholaminergic systems [13]. For example, micro-injection of vasopressin into the dentate gyrus of rats has been shown to improve memory and to accelerate noradrenaline turnover in this region [14]. It has been suggested [15] that differences in the tonic influence of vasopressinergic fibres may contribute to differences in learning as well as in catecholaminergic turnover between individual animals.

An index of sensitivity of catecholamine receptors is the accumulation of cyclic AMP in brain slices in response to noradrenaline. Schneider *et al.* [18] showed that IP administration of desglycyl-8-arginine vasopressin (DGAVP) to mice significantly elevated cyclic AMP levels after 1 hour in 5 regions of the brain and after 24 hr in 10 regions of the brain. Courtney and Raskind [4] showed potentiation of the stimulatory effect of dopamine on adenylate cyclase by vasopressin at 1–100 μM in rat caudate homogenates, although there was no direct effect of the peptide on enzyme activity. In the homogenate preparation synaptic connections are absent and no neuromodulatory or other indirect actions of peptides can be expressed. In the present work, therefore, the effects of lysine vasopressin (LVP), arginine vasopressin (AVP), and the analog 1-desamino-8-D-arginine vasopressin (DDAVP), on noradrenaline-sensitive cyclic AMP accumulation were studied in rat brain slices. This preparation contains intact cells and is thus suitable for investigation of indirect modulatory as well as direct effects. DDAVP was used since it possesses little pressor but high antidiuretic activity, and is one of the most potent vasopressin analogs in stimulating adenylate cyclase in kidney [8].

In addition, cyclic AMP responses of brain slices to noradrenaline were determined in rats which had received acute or chronic DDAVP in vivo. The results were compared with the effects of acute and chronic administration of DDAVP on initial exploratory activity of rats in an open-field apparatus. The experiments were then repeated using rats which had received IP desipramine and intracisternal 6-hydroxydopamine (6-OHDA) on the 5th day of life. This treatment destroys brain dopamine but not noradrenaline stores [19,20], and has been shown to result in persistent deficits in shuttle-box learning which are vastly improved by treatment with a vasopressin analog [10]. The functional deficiency of brain dopamine in these animals has also been suggested as a possible model for the group of childhood attention and learning disorders know as minimal brain dysfunction [19]. Work in our laboratory has suggested that DDAVP is effective in treatment of these disorders [7], although its mechanism of action is unknown. Cyclic AMP has been proposed to be involved in processes of learning and memory [3,17]. Since vasopressin affects dopamine stimulation of cyclic AMP production [4], vasopressin treatment of desipramine and 6-OHDA pretreated rats enabled investigation of the cyclic AMP response independently of its interaction with dopamine. This design allowed for specific expression of noradrenaline effects on vasopressin induced changes in cyclic AMP production.

TABLE 1

EFFECT OF IN VITRO ADDITION OF VASOPRESSIN ANALOGS ON CYCLIC AMP LEVELS IN BRAIN SLICES

Addition	Cortex	Caudate	Hippocampus
None	10.2 ± 2.2 (8)	14.0 ± 2.8 (8)	9.3 ± 1.4 (10)
LVP, 0.2 μM	n.d.	n.d.	22.1 ± 5.2 (7)
AVP, 28 nM	10.3 ± 1.6 (5)	n.d.	8.8 ± 1.0 (7)
50 μM NA	27.9 ± 3.1 (9)	26.7 ± 5.2 (8)	31.7 ± 3.2 (14)
50 μM NA +AVP 28 nM	15.7 ± 3.1 (6)‡	24.8 ± 5.0 (8)*	33.6 ± 6.7 (8)
DDAVP 0.56 μM	6.9 ± 2.3 (4)	n.d.	13.6 ± 4.2 (4)
50 μM NA +DDAVP 0.56 μM	18.3 ± 3.7 (4)†	n.d.	27.4 ± 8.0 (4)

All data expressed as pmol cyclic AMP/mg protein, mean ± S.E.M.
Significantly different from NA alone by Student's t test, ‡$p<0.01$, †$p<0.05$, and *$p<0.05$ when results expressed as % of corresponding basal values.
n.d. = not determined.

METHOD

Treatment of Animals

Male albino pups (Sabra strain) 5 days old were injected IP with desipramine (200 μg/rat in 25μl) followed 1 hour later by intracisternal (IC) 6-OHDA (100 μg/rat in 25 μl of 0.4% ascorbic acid in sterile saline) or with vehicle only. Pre-injection of desipramine is designed to spare noradrenaline neurons and to create a dopamine specific lesion. Rats were separated from their mothers at 28 days of age and were re-distributed into new groups of 8–9 rats/group. SC treatments with DDAVP, 20 μg/rat, or saline, 0.5 ml/rat (body weight 180–250 g) started at the age of 2 months and were given 3 times per week (on the 1st, 3rd and 5th days of the week). Three treatment regimens were used: (1) chronic treatment with DDAVP for 3 months; (2) treatment with saline for 3 months, and (3) treatment with DDAVP only during the first week, once on the first day of open field study in the 7th week, and once again before sacrifice. On the DDAVP-free days the rats were injected with saline. Altogether 6 groups of rats were studied; three IC 6-OHDA pretreated groups and three IC vehicle pretreated groups. Treatments were given at 9.00 a.m. or one hour before behavioural testing or sacrifice. The rats in group (3) are described as acutely treated to emphasize the contrast with those which received DDAVP continuously for a period of 3 months.

Open-Field Tests

Behavioural tests were performed in the second half of the dark period of the dark-light cycle (dark from 05.00 till 17.00). The open field was a square wooden box 100×100 cm. White lines divided the black floor into 25 equal squares (20×20 cm.). Illumination was provided by a 40-w bulb. The animals were placed individually in the corner of the open field one hour after treatment. The number of squares crossed every 2 min over 10-min periods were counted. Counting started after the first 2 min. An "event" was recorded whenever an animal placed all four legs over a new square. Observations were performed on the 1st, 3rd and 5th day of the 7th week of treatment.

Tissue Slices and Cyclic AMP determination

Brain areas were dissected on a cooled dish and sliced using a McIlwain tissue chopper set at 0.35 mm. All slices from each area were pre-incubated for 30 min at 37°C in 10 ml Krebs-Ringer's bicarbonate buffer containing 1.29 mM $CaCl_2$, gassed with 95% O_2:5% CO_2. The slices were then collected on a Buchner funnel and distributed among vials containing 5 ml Krebs-Ringer's with or without noradrenaline for 20 min incubation. In experiments on the in vitro effects of vasopressin, small amounts of AVP or LVP (Sigma Chem. Co.) or DDAVP (Ferring AB, Sweden) diluted in Krebs-Ringer's solution, were added simultaneously with the noradrenaline to give the desired final concentrations. After this period the slices were transferred to test-tubes, centrifuged, the medium decanted, and the pellets homogenized in 1 ml (for cortex) or 0.5 ml (for caudate and hippocampus) 95% ethanol. Aliquots of the supernatants were evaporated to dryness under N_2 and cyclic AMP determined by displacement of ^{3}H-cAMP (Radiochemical Centre, Amersham) using a protein binding method based on that of Brown *et al.* [1].

RESULTS

In vitro addition of any of the vasopressin analogs had no direct effect on basal cyclic AMP levels in any of the three brain regions studied (Table 1). Addition of 50 μM noradrenaline produced a 3-fold rise in cyclic AMP levels in cortex and hippocampus and a 2-fold rise in caudate. The effect of noradrenaline in cortex was significantly reduced both by 28 nM AVP ($p<0.01$) and 0.56 μM DDAVP ($p<0.05$). The concentrations at which these analogs were tested were based on the 20:1 ratio reported for their behavioral activities [21]. In caudate slices, AVP at the same concentration inhibited

TABLE 2

EFFECT OF IN VIVO ADMINISTRATION OF DDAVP TO VEHICLE PRETREATED RATS ON CYCLIC AMP LEVELS IN BRAIN SLICES

Brain Area and Treatment of Slices	Chronic DDAVP	Control	Acute DDAVP
Cortex, basal	25.0 ± 7.5 (6)	16.3 ± 7.1 (6)	15.0 ± 5.6 (5)
+ 50 μM NA	36.5 ± 7.8 (6)	33.5 ± 12.6 (6)	23.9 ± 9.4 (5)
Diff, NA-basal	11.5 ± 4.8 (6)	17.2 ± 5.7 (6)	8.9 ± 4.0 (5)
Caudate, basal	24.0 ± 8.1 (6)	14.4 ± 3.6 (6)	18.8 ± 11.5 (6)
+ 50 μM NA	28.9 ± 8.1 (6)	38.0 ± 16.7 (6)	25.6 ± 8.1 (6)
Dif, NA-basal	4.9 ± 4.9 (6)	24.5 ± 12.2 (6)	6.8 ± 5.3 (6)
Hippocampus, basal	23.9 ± 10.8 (5)	21.4 ± 7.3 (5)	17.3 ± 6.7 (5)
+ 50 μM NA	34.9 ± 16.0 (5)	40.9 ± 11.1 (5)	31.7 ± 19.7 (5)
Diff, NA-basal	10.9 ± 5.6 (5)	19.5 ± 8.7 (5)	14.4 ± 13.2 (5)

Data expressed as pmol cyclic AMP/mg protein, mean ± S.E.M. of no. of animals in parentheses.

Differences between basal and stimulated activity in each experiment, were averaged to obtain the *mean* differences given above.

TABLE 3

EFFECT OF IN VIVO ADMINISTRATION OF DDAVP TO 6-OH DA-TREATED RATS ON CYCLIC AMP LEVELS IN BRAIN SLICES

Brain Area and Treatment of Slices	Chronic DDAVP	Control	Acute DDAVP
Cortex, basal	8.6 ± 2.6 (6)†	20.6 ± 4.5 (6)	7.1 ± 1.0 (3)*
+ 50 μM NA	22.1 ± 4.0 (6)†	51.9 ± 11.7 (6)	18.7 ± 3.1 (3)*
Diff, NA-basal	13.4 ± 3.7 (6)*	31.3 ± 8.2 (6)	11.4 ± 2.8 (3)*
Caudate, basal	13.4 ± 6.5 (4)	24.3 ± 9.2 (4)	12.6 ± 4.0 (3)
+ 50 μM NA	28.4 ± 13.1 (4)	57.4 ± 19.1 (3)	11.3 ± 5.8 (3)*
Diff, NA-basal	11.1 ± 6.3 (3)	26.9 ± 10.4 (3)	
Hippocampus, basal	14.8 ± 5.7 (5)	13.5 ± 3.8 (5)	10.3 ± 4.1 (3)
+ 50 μM NA	12.4 ± 3.1 (6)*	31.5 ± 9.6 (6)	27.1 ± 8.3 (3)§
Diff, NA-basal	5.6 ± 3.3 (5)‡	22.7 ± 6.6 (5)	16.9 ± 3.5 (3)§

Data expressed as pmol cyclic AMP/mg protein, mean ± S.E.M. of no. of animals in parentheses.

Significantly different by Student *t*-test vs. control, *$p<0.05$, †$p<0.025$, ‡$p<0.01$, vs. chronically treated rats, §$p<0.05$.

Difference scores were computed as in Table 2.

the effect of noradrenaline to a lesser degree, ($p<0.05$ when results expressed as percentage of corresponding basal values) while in hippocampal slices there was no significant effect of any of the analogs tested on either basal or noradrenaline-stimulated activities.

After acute or chronic treatment with DDAVP, rat cortex or hippocampal slices showed a slightly reduced response to noradrenaline compared to slices from saline treated rats, while in caudate the effect was much greater, although not reaching statistical significance (Table 2). When rats were pretreated with desipramine and 6-OHDA before beginning injections of DDAVP (Table 3), both basal and noradrenaline-stimulated cyclic AMP levels in cortex were significantly lower in rats which had received either acute ($p<0.05$) or chronic ($p<0.025$) DDAVP, compared to controls. In caudate slices the response to noradrenaline was vastly reduced ($p<0.05$) in rats which had received acute DDAVP, so that no increment above basal could be detected. The response in chronically treated rats was also reduced, but to a lesser degree (difference not significant). In hippocampus there was a significant ($p<0.05$) reduction in the noradrenaline response in slices from chronically treated rats only. The response in hippocampal slices from acutely treated rats did not differ from that in controls, but was significantly ($p<0.05$) greater than that in chronically treated rats. The response to noradrenaline in cortical slices from control rats (33.5±12.6 p mol/mg protein, Table 2) was increased following 6-OHDA treatment (to 51.9±11.7 p mol/mg protein,

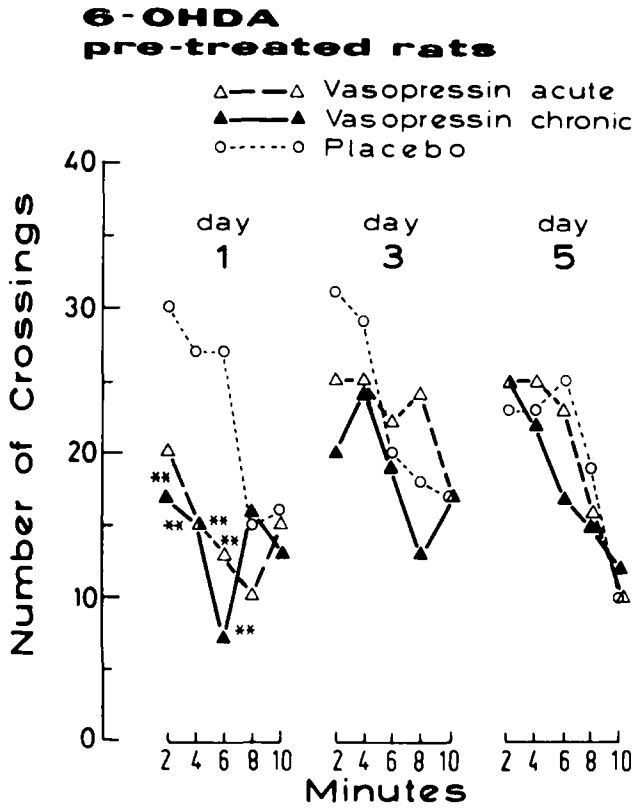

FIG. 1. Open field behaviour of 6-OHDA treated rats shown as mean number of crossings in each of the five 2-min periods of the three daily sessions for each group. Comparisons between vasopressin treated and placebo groups by Student's t test, $^{**}p<0.01$, $^{***}p<0.005$.

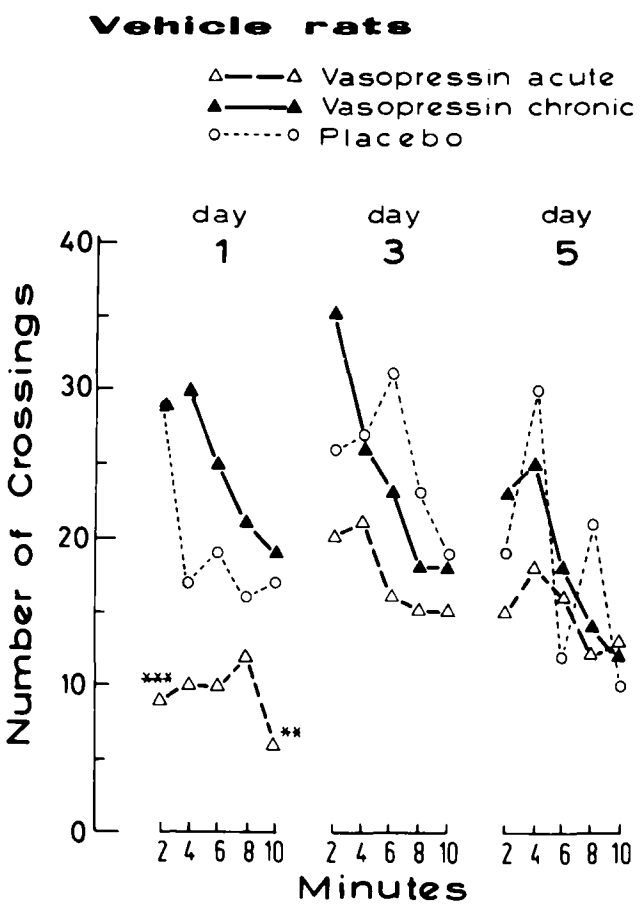

FIG. 2. Open-field behaviour of vehicle treated rats, as Fig. 1.

Table 3), suggesting a partial supersensitivity due to catecholamine depletion.

Open-field behavioural observations in 6-OHDA pretreated rats showed reduced crossings per 10 min after both acute and chronic DDAVP on the first day of testing only (Fig. 1). In vehicle pretreated animals, a reduced number of crossings was observed only in the acutely treated animals (Fig. 2). There were no significant differences between 6-OHDA- and vehicle-pretreated rats except in the case of animals which received chronic DDAVP. In this group vehicle pretreated animals showed more crossings for the first 6 min ($p<0.05$) than 6-OHDA pretreated animals.

The number of rearings was not reduced by DDAVP treatment on any day of testing. After 10 min in the open field, however, fewer boli were detected in animals receiving DDAVP.

DISCUSSION

The present results confirm the absence of a direct effect of vasopressin on adenylate cyclase in rat brain homogenates, as found by Courtney and Raskind [4], but indicate clear inhibition of the noradrenaline response, both in vitro and in vivo. Several explanations for this are possible. An indirect effect via the pressor action of vasopressin is unlikely, since DDAVP possesses only slight pressor activity. Noradrenaline-induced cyclic AMP accumulation was found to be decreased in the hypothalamus of hypertension-prone rats by Kobrin *et al.* [12], but these authors found no such difference between normotensive and hypertensive rats in cortex, in contrast to the decrease found in DDAVP-treated rats in the present study. An indirect action via release of corticosteriods, which reduce the sensitivity of noradrenaline-sensitive adenylate cyclase in brain [16], could result from the ACTH-releasing action of DDAVP [11]. However, the in vitro effects observed with both vasopressin analogs argue against mediation by other hormones. Release of endogenous noradrenaline, which has been shown in the dentate gyrus after microinjection of vasopressin, would account for the reduced response to added noradrenaline after chronic treatment by a subsensitivity mechanism. Mechanisms other than a presynaptic action on noradrenergic nerve terminals may however be involved, since in one study [9] LVP failed to alter noradrenaline uptake or release in the hippocampus.

It was recently reported by Church [2] that in vitro addition of LVP to mouse hippocampal slices had no effect on basal cyclic AMP levels, but potentiated the stimulatory effect of noradrenaline. The discrepancy with the results reported in this study could be partially attributed to species differences or to the higher concentration of LVP used by Church (1 μM). The contrast with the results of Schneider *et al.* [18] may also be related to species or dose differences. In addition, DGAVP, the analog used by these authors, is devoid of antidiuretic activity.

It is of interest that DDAVP was 20 times less potent than

AVP in behavioural activity as determined by the pole-jumping avoidance test [21]. The affinity of DDAVP for activation of adenylate cyclase in human kidney membranes, and for displacement of labelled vasopressin in binding studies, was also 5–10 fold less than that for AVP. The use of DDAVP at a concentration 20 times greater than that employed for AVP in the present study would therefore seem to be justified. LVP shows 63% of the behavioural potency of AVP [21] yet also gives a 10-fold decrease in affinity for activation of cyclase or displacement of binding [8].

The open-field behavioural observations in the present study are similar to results obtained by Crine [5] after LVP treatment, in which fewer crossings were observed on the first day of testing compared to the control groups, but no differences between the treatments were noted on repeated observation. The reduced number of crossings seen after DDAVP cannot be explained by an effect on locomotion, since it was not seen on repeated testing. In contrast, DDAVP reduced the number of boli on each day of administration. There was no apparent correlation between emotionality, as reflected in the number of boli, and the number of crossings. Open-field activity may thus be regarded as exploratory behaviour connected with learning of a new environment, and not as an expression of emotionality.

A correlation between the effects of vasopressin treatment on noradrenaline-induced cyclic AMP accumulation and its actions on learning and memory is suggested by the observation that in vehicle treated animals, acute injection of DDAVP was more effective than chronic injection in reducing both the noradrenaline response in cortex and the initial exploratory activity in the open-field apparatus. In 6-OHDA treated animals, acute and chronic injections were equally effective in reducing both the noradrenaline response in cortex and initial exploratory activity. The magnitude of the effects on the noradrenaline response obtained with DDAVP were also in general greater in 6-OHDA treated animals, in keeping with the greater effects on shuttle-box learning observed when baseline levels were reduced by the dopamine depletion [10]. A further link between the effects reported here and the active avoidance learning model is provided by the fact that the caudate nucleus, which was the brain area most susceptible to the DDAVP-induced reduction in the noradrenaline response (Table 3) was the only brain region out of the 4 tested [10] to show a correlation between vasopressin levels and the conditioned avoidance response. The independence of the dopamine and vasopressin effects on learning has been demonstrated previously [10]. The effects of noradrenaline in the present study and the increased responses to DDAVP shown when animals were pre-treated with 6-OHDA and desipramine so as to destroy the dopaminergic system but leave the adrenergic system intact, provide further evidence for the involvement of the adrenergic system in the central effects of vasopressin [13].

REFERENCES

1. Brown, B. L, J. D. M. Albano, R. P. Ekins, A. M. Sgherzi and W. Tampion. A simple and sensitive saturation assay method for the measurement of adenosine 3:5′-cyclic monophosphate. *Biochem J* **121:** 561–562, 1971.
2. Church, A. C. Vasopressin potentiates the stimulation of cyclic AMP accumulation by norepinephrine. *Peptides* **4:** 261–263, 1983.
3. Chute, D. L., J. W. Villiger and N. F. Kirton. Testing cyclic AMP mediation of memory: reversal of α-methyl-p-tyrosine-induced amnesia. *Psychopharmacology (Berlin)* **74:** 129–131, 1981.
4. Courtney, N. and M. Raskind. Vasopressin affects adenylate cyclase activity in rat brain: a possible neuromodulator. *Life Sci* **32:** 591–596, 1983.
5. Crine, A. F. Effects of vasopressin on open-field behaviour in rats. *Physiol Psychol* **9:** 109–113, 1981.
6. de Wied, D. Behavioural actions of neurohypophysial peptides. *Proc R Soc Lond (Biol)* **210:** 183–195, 1980.
7. Eisenberg, J., S. Chazan-Gologorsky, J. Hattab and R. H. Belmaker. A controlled trial of vasopressin treatment of childhood learning disorders. *Biol Psychiatry* **19:** 1137–1141, 1984.
8. Guillon, G., D. Butlen, B. Cantau, T. Barth and S. Jard. Kinetic and pharmacological characterization of vasopressin membrane receptors from human kidney medulla; relation to adenylate cyclase activation. *Eur J Pharmacol* **85:** 291–304, 1982.
9. Hagan, J. J. and D. J. K. Balfour. Lysine vasopressin fails to alter (^{3}H)-noradrenaline uptake or release from hippocampal tissue in vitro. *Life Sci* **32:** 2517–2522, 1983.
10. Hamburger-Bar, R., R. P. Ebstein and R. H. Belmaker. Vasopressin effect on learning in 6-OHDA pre-treated rats: correlation with caudate vasopressin levels. *Biol Psychiatry* **19:** 735–743, 1984.
11. Knepel, W., L. Homalka and M. Vlaskovska. In vitro CRF activity of vasopressin analogs is not related to pressor activity. *Eur J Pharmacol* **91:** 115–118, 1983.
12. Kobrin, I., R. B. Ebstein and D. Ben-Ishay. Cyclic AMP generation in hypothalamus of hypertension-prone and -resistant rats. *Clin Sci* **59:** 247 s–249 s, 1980.
13. Kovacs, G. L., B. Bohus and D. H. G. Versteeg. The interaction of posterior pituitary neuropeptides with monoaminergic neurotransmission: significance in learning and memory processes. *Prog Brain Res* **53:** 123–140, 1980.
14. Kovacs, G. L., B. Bohus, D. H. G. Versteeg, E. R. de Kloet and D. de Wied. Effect of oxytocin and vasopressin on memory consolidation: sites of action and catecholaminergic correlates after local microinjection into limbic-midbrain structures. *Brain Res* **175:** 303–314, 1979.
15. Kovacs, G. L., D. H. G. Versteeg, E. R. de Kloet and B. Bohus. Passive avoidance performance correlates with catecholamine turnover in discrete limbic brain regions. *Life Sci* **28:** 1109–1116, 1981.
16. Mobley, P. L., D. H. Manier and F. Sulser. Norepinephrine-sensitive adenylate cyclase system in rat brain; role of adrenal corticosteroids. *J Pharmacol Exp Ther* **226:** 71–77, 1983.
17. Randt, C. T., M. E. Judge, K. A. Bonnet and D. Quatermain. Brain cyclic AMP and memory in mice. *Pharmacol Biochem Behav* **17:** 677–680, 1982.
18. Schneider, D. R., B. T. Felt and H. Goldman. Desglycyl-8-arginine vasopressin affects regional mouse brain cyclic AMP content. *Pharmacol Biochem Behav* **16:** 139–143, 1982.
19. Shaywitz, B. A., R. D. Yager and J. H. Klopper. Selective brain dopamine depletion in developing rats; an experimental model of minimal brain dysfunction. *Science* **191:** 305–308, 1976.
20. Smith, R. D., B. R. Cooper and G. R. Breese. Growth and behavioral changes in developing rats treated intracisternally with 6-hydroxydopamine; evidence for involvement of brain dopamine. *J Pharmacol Exp Ther* **185:** 609–619, 1973.
21. Walter, R., J. M. van Ree and D. de Wied. Modification of conditioned behavior of rats by neurohypophyseal hormones and analogues. *Proc Natl Acad Sci USA* **75:** 2493–2496, 1978.