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Release of vasopressin and oxytocin by excitatory amino acid agonists and the effect of antagonists on release by muscarine and hypertonic saline, in the rat *in vivo*

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1 It has been claimed that glutamate is the dominant excitatory neurotransmitter in neuroendocrine regulation. The evidence is derived mainly from *in vitro* experiments.

2 We have investigated *in vivo* a possible role of excitatory amino acids (EAAs) in the neural control of release of vasopressin (AVP) and oxytocin from the neurohypophysis.

3 In rats under ethanol anaesthesia in which a diuresis was maintained by a constant fluid load, the i.c.v. injection of glutamate and the synthetic agonists α-amino, 3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) and N-methyl-D-aspartate (NMDA) produced an antidiuretic response (ADR) which was abolished by an AVP antagonist. For AMPA and NMDA it was shown that this ADR was accompanied by increased urinary excretion of AVP and oxytocin.

4 The selectivity of antagonists was tested in this system. D-2-Amino-5-phosphonopentanoate (D-AP5) blocked the responses to NMDA but not to AMPA; 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX) blocked the responses to both agonists.

5 The ADR to muscarine and hypertonic saline i.c.v., and the increase in excretion of AVP and oxytocin in response to muscarine, were blocked by CNQX but not by D-AP5.

6 The results suggest that hypertonic saline releases AVP and muscarine releases both AVP and oxytocin, at least in part, by activating a glutaminergic input to the SON and PVN involving an AMPA receptor. This input could function as a terminal interneurone in afferent neural pathways to these nuclei.

Keywords: Neurohypophysis; vasopressin; oxytocin; muscarine; noradrenaline; hypertonic saline; excitatory amino acid agonists and antagonists

Introduction

There is increasing evidence that glutamate or another endogenous excitatory amino acid (EAA) acts as a transmitter in the neural control of release of vasopressin (AVP) and oxytocin from the neurohypophysis. The magnocellular neurosecretory cells (MNCs) in the supraoptic and paraventricular nuclei (SON and PVN) which secrete these hormones, express two types of ionic glutamate receptors characterized by the synthetic agonists AMPA (α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate) and NMDA (N-methyl-D-aspartate) and their respective antagonists CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and D-AP5 or DL-AP5 (2-amino-5-phosphonopentonoate) (Bourque *et al.*, 1995). Immunocytochemistry at the ultrastructural level has revealed the presence of glutaminergic axon terminals in the SON and PVN in synaptic contact with the dendrites and perikarya of the MNCs secreting both AVP and oxytocin (Meeker *et al.*, 1988; 1993; Van de Pol *et al.*, 1990). Electrical stimulation of a region dorsolateral to the SON (Gribkoff, 1991) or lateral to the PVN (Van de Pol *et al.*, 1990), in hypothalamic slices, evokes excitatory postsynaptic potentials (e.p.s.ps) in identified MNCs within the nuclei which are inhibited by CNQX and D-AP5.

The majority of the afferent neural inputs to the SON and PVN from peripheral receptors or other parts of the CNS which control the release of AVP and oxytocin, do not directly innervate the MNCs in these nuclei but terminate in the surrounding 'perinuclear zone (PNZ)' (Tribollet *et al.*, 1985; Raby & Renaud 1989; Anderson *et al.*, 1990; Renaud *et al.*, 1993). The transmission of afferent nerve impulses from the axon terminals in the PNZ to the MNCs must involve an interneurone. The glutaminergic input could subserve this function. It is possible, also, that putative neurotransmitters or other pharmacologically active substances which cause release of AVP and oxytocin on i.c.v. injection in the rat could act indirectly by stimulating this glutaminergic input.

Release of AVP has been demonstrated *in vitro* by incubating hypothalamic slices with NMDA and kainate which were blocked by DL-AP5 and CNQX respectively (Costa *et al.*, 1992). We have now investigated the effect of EAA agonists on the release of AVP and oxytocin *in vivo*. EAA agonists and antagonists were adminstered centrally by i.c.v. injection in rats in which a diuresis was induced by water-loading under ethanol anaesthesia. The advantage of the *in vivo* method is that release of AVP can be detected instantly by the appropriate biological response, that is an inhibition of urine flow or 'antidiuretic response (ADR)'. Release of both hormones is quantitated subsequently by measuring the rates of urinary excretion of AVP and oxytocin during the ADR. This model has been validated in previous work (Bisset *et al.*, 1990; 1992). To test the possibility that drugs administered i.c.v. cause release of AVP and oxytocin by stimulating glutaminergic interneurones, we have also tested the effect of EAA antagonists on release of these hormones by muscarine and hypertonic saline.

Methods

The experiments were carried out on male Wistar rats, 220–300 g, under ethanol anaesthesia. A diuresis was induced by administering through a stomach tube, 50 ml kg^{-1} 12%

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ethanol, followed after 30 min by 30 ml kg^{-1} tap water. An i.v. infusion of isotonic glucose saline solution was delivered through a feedback system to keep the body weight, and hence the fluid load, constant. Anaesthesia was maintained by adding 1–3% ethanol to the perfusion fluid. Urine flow was recorded by means of a drop counter set to a 1 min time base and arterial blood pressure from a femoral artery. Drugs were injected in a volume of 1–4 μl into a cannula in a lateral cerebral ventricle (i.c.v.). Samples of urine (5 ml) were collected during an initial control period and immediately following drug injections. The samples were extracted with octadecasilyl silica. AVP and oxytocin-like-radioimmunoreactivity (OLRI) were estimated in the extracts by radioimmunoassay. The rates of excretion of urine and of the hormones were calculated over the period required to collect the 5 ml samples. An antidiuretic response (ADR) was measured as the reduction in the rate of urine flow during this period expressed as a percentage of the control flow immediately preceding the injection. Full experimental details of the preparation and the extraction and the assay methods have been published (Bisset *et al.*, 1990).

Drugs

The EAA agonists and antagonists, muscarine, noradrenaline and sodium nitroprusside were obtained from Sigma. The AVP antagonist (D-$[CH_2]_5$D-Tyr [Et] valyl arginine vasopressin VAVP (Sawyer *et al.*, 1981) was a generous gift from Dr Manning of the Department of Biochemistry, Medical College of Ohio, U.S.A. CNQX was supplied in a vial containing 10 mg. This was dissolved in 1 ml dimethylsulphoxide (DMSO) to give a 40 mM solution. Further dilutions were made with artificial CSF. The highest concentration of CNQX tested as an antagonist was 10 mM. A 1:4 dilution of DMSO tested under the same experimental conditions as CNQX did not cause an ADR nor did it inhibit the ADR and increase in urinary excretion of AVP and OLRI produced by muscarine.

Statistics

Results are expressed as mean ± standard error of mean (s.e.mean). Significance of differences between means was determined by Students' *t* test, paired or unpaired, as appropriate.

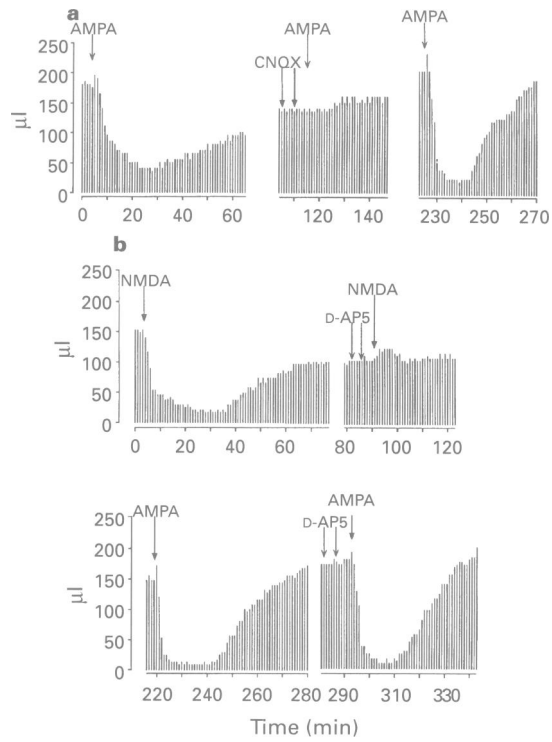

Figure 1 Urine flow in two water-loaded rats (a and b) under ethanol anaesthesia. Each vertical line in the traces shows the flow (μl) in 1 min and the time course of the experiment is indicated on the horizontal axis. Each section records the flow during the collection of a 5 ml urine sample after the injection of an agonist. (a) Antidiuretic response (ADR) to AMPA and reversible inhibition by CNQX. The traces show the ADRs to 3 injections of AMPA, 0.8 nmol, i.c.v. The first served as a control; 2 injections of CNQX 40 nmol i.c.v. were given 10 min and 5 min before the second injection of AMPA and the third was given 120 min after the first of the 2 injections of CNQX. (b) ADRs to NMDA 4 nmol and AMPA 0.4 nmol i.c.v.: selective inhibition of the ADR to NMDA by D-AP5. The first injection of each agonist served as a control: D-AP5 4 nmol i.c.v. was injected 10 min and 5 min before the second injection.

Results

EAA agonists and antagonists

On i.c.v. injection in water-loaded rats, glutamate, AMPA and NMDA produced graded, reversible ADRs which could be repeated consistently in the same rat without tachyphylaxis. The minimum effective dose to produce a measurable ADR ranged from 40–1600 nmol (median 400 nmol: $n=7$) for glutamate, 0.1–0.8 nmol (median 0.4 nmol: $n=74$) for AMPA and 1–8 nmol (median 2 nmol: $n=47$) for NMDA. Typical ADRs to AMPA and NMDA are illustrated in Figure 1. The ADR to each agonist was accompanied by a rise in blood pressure. The ADR, but not the pressor response, was abolished by the AVP antagonist D-$[CH_2]_5$ D-Tyr [Et] VAVP which blocks both responses to AVP (Bisset *et al.*, 1992). Figures 2 and 4c show that the ADR to AMPA and NMDA was accompanied by highly significant increases in the urinary rates of excretion of both AVP and OLRI.

To test the potency and selectivity of CNQX as an EAA antagonist, two injections of CNQX were given, the first 10 min and the second 5 min before the agonist. This is similar to the procedure which was found to be effective in previous work with nicotine and neosurugatoxin (Bisset *et al.*, 1992). It allows the antagonist to be given in divided doses ensuring adequate absorption and distribution before agonist injection. In two of four experiments, two injections of 20 nmol CNQX caused only partial or no inhibition of the response to AMPA. The lowest dose to produce a total and consistent inhibition was 40 nmol. Figure 1a illustrates the total and reversible inhibition of the ADR to 0.8 nmol AMPA by 2 doses of 40 nmol CNQX. The results of five experiments are shown in Figure 2. Control injections of AMPA produced large ADRs and increases in the urinary excretion of AVP and OLRI. Ten min after the first of two injections of 40 nmol CNQX (20 nmol in one experiment), the ADR was abolished and the rates of excretion of AVP and OLRI reduced to the basal levels at the beginning of the experiment. A partial recovery occurred 60 min after CNQX and a significantly greater recovery at 120 min. The recovery of the ADR and AVP excretion was incomplete. In six of seven experiments two doses of 40 nmol CNQX injected 10 min and 5 min before NMDA 1–4 nmol blocked the ADR. In one experiment the rates of excretion of AVP and OLRI were reduced to basal levels 10 min after the first injection: at 60 min there was almost complete recovery. In six experiments in which CNQX blocked the ADR to AMPA, there was no significant inhibition of the ADR to hypotension induced by 200 μg sodium nitroprusside i.v.; the mean ADR was 48.5 ± 3.2% before, and 45.7 ± 4%, after CNQX. These results showed that, although CNQX was not selective for the AMPA receptor, it did not cause a non-specific depression of the AVP-secreting cells in the SON and PVN.

The antagonist D-AP5 was highly selective for NMDA. This was shown in five experiments, one of which is illustrated in Figure 1. Two injections of AMPA and NMDA were given to the same rat, the first serving as a control. The same procedure was followed for D-AP5 as for CNQX. Two injections of

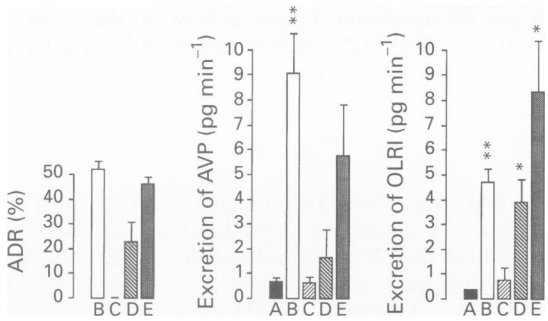

Figure 2 Results of 5 experiments on water-loaded rats under ethanol anaesthesia. The effect of AMPA on urine flow (ADR) and urinary excretion of vasopressin (AVP) and oxytocin-like radioimmunoreactivity (OLRI): reversible inhibition by CNQX. Four injections of AMPA (0.2–0.8 nmol: see Methods) were given i.c.v. to each rat. The first (B) served as a control, CNQX 40 nmol (in one experiment 20 nmol) was injected i.c.v. 10 min and 5 min before the second injection (C). The third (D) and fourth (E) injections of AMPA were given 60 min and 120 min respectively, after the first injection of CNQX. A control sample of urine (A) was collected from each rat at the start of the experiment to estimate basal rates of excretion of AVP and OLRI. The asterisks above the columns indicate a significant difference in rate of excretion compared with that in the control sample (*$P<0.05$; **$P<0.01$).

4 nmol D-AP5 were given 10 min and 5 min before the second injection of the agonist. The mean ADR to control injections of NMDA 4 nmol was $41\pm4.8\%$: in every experiment the ADR was abolished by D-AP5 and the mean rates of excretion of AVP and OLRI were not significantly different from the basal levels. AMPA 0.2–0.8 nmol was not inhibited by D-AP5. The mean ADR and the rates of excretion of AVP and OLRI were $44\pm3.5\%$, 4.3 ± 0.58 pg ml^{-1} and 4.8 ± 0.95 pg ml^{-1}, respectively, before, and $40\pm6.1\%$, 5.5 ± 1.03 pg ml^{-1} and 9.1 ± 1.2 pg ml^{-1}, after the antagonist. In two experiments, the time course of inhibition of NMDA by D-AP5 was tested; the ADR and excretion of AVP had returned to at least 60% of the control level 60 min after the injection of D-AP5 and the OLRI had recovered completely.

The effect of EAA antagonists on the response to muscarine and hypertonic saline (HS)

In all experiments, a standard dose of 40 nmol CNQX or 4 nmol D-AP5 was injected 10 min and 5 min before the agonist.

The effect of CNQX on the ADRs to muscarine (a) and HS (b) is shown in Figure 3. Two injections of the agonist were given, the first serving as a control and the second after CNQX. The ADR to both agonists was abolished. For comparison, the effect of CNQX on the ADR to noradrenaline (NA) is illustrated in Figure 3c. Since this response had been shown in previous work (Bisset & Fairhall, unpublished) to exhibit tachyphylaxis, only one dose of NA could be tested in a single rat. In the experiment of Figure 3c, a control injection of AMPA 0.1 nmol was given. NA after CNQX produced a large and prolonged ADR; a second injection of AMPA given after the collection of the 5 ml sample following NA, and without further CNQX, produced no ADR. This demonstrated that NA produced an ADR during an effective and prolonged block of AMPA receptors. A similar result was obtained in 5 other experiments. The lack of an inhibitory effect of CNQX on the ADR to NA shows that inhibition of the ADR to muscarine and HS (Figure 3a, b) is not due to a non-specific depression of the AVP-secreting cells in the SON and PVN.

The results of three series of experiments with muscarine are summarised in Figure 4. In each series, and in confirmation of

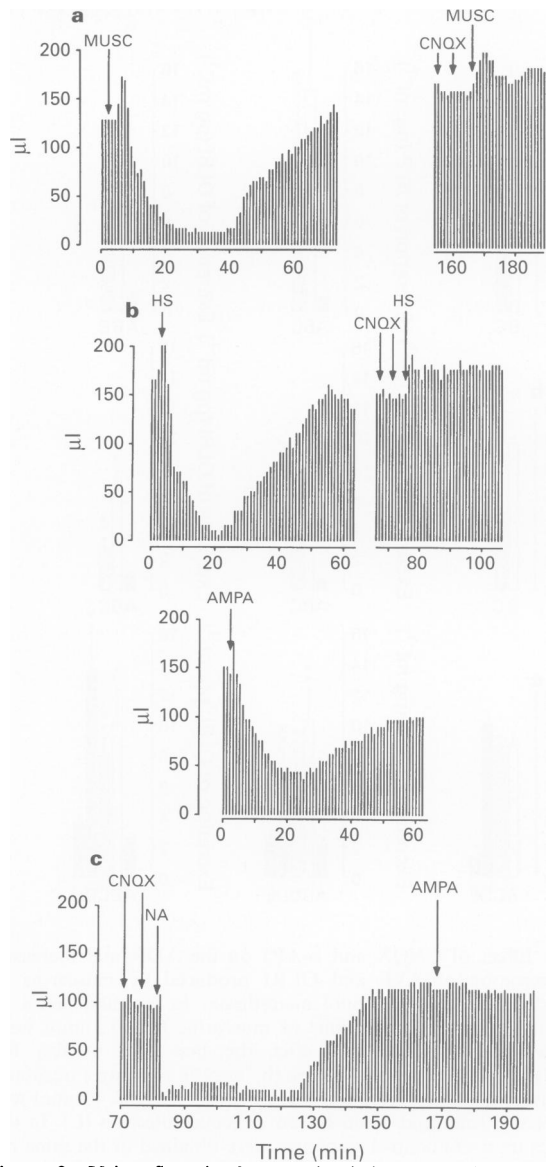

Figure 3 Urine flow in 3 water-loaded rats (a, b and c) under ethanol anaesthesia recorded as in legend to Figure 1. The effect of CNQX on the ADRs to muscarine 0.1 nmol (20 ng) (Musc) (a) hypertonic saline (HS) 6 μmol (b) and noradrenaline (NA) 0.1 μmol (20 μg) (c) i.c.v. In (a) and (b) a control injection of the agonist was given: CNQX 40 nmol was injected i.c.v. 10 min and 5 min before a repeat injection of the agonist. In (c) a control response to AMPA 0.1 nmol was obtained: CNQX 40 nmol was injected i.c.v. 10 min and 5 min before NA and, after the collection of a 5 ml urine sample following NA, AMPA was repeated without any further injection of CNQX.

previous work (Bisset *et al.*, 1992), the ADR was accompanied by an increase in the excretion of AVP and OLRI. In the first series of experiments (a), two successive injections of muscarine were given at an interval of 60 min. The absence of a significant difference in the responses excludes tachyphylaxis. In the other two series of experiments, therefore, two injections of muscarine were given to each rat, the first serving as a control. Figure 4b shows that CNQX produced a significant reduction in the ADR to muscarine and in the rates of excretion of AVP and OLRI. The rate of excretion of AVP did not differ significantly from the control level. Since CNQX was shown to be a non selective antagonist of AMPA receptors, a series of experiments was carried out (Figure 4c) to test the effect of D-AP5 on the response to muscarine. Each rat was given two injections of muscarine and two of NMDA, one before and one after, D-AP5. D-AP5 abolished the ADR to NMDA in

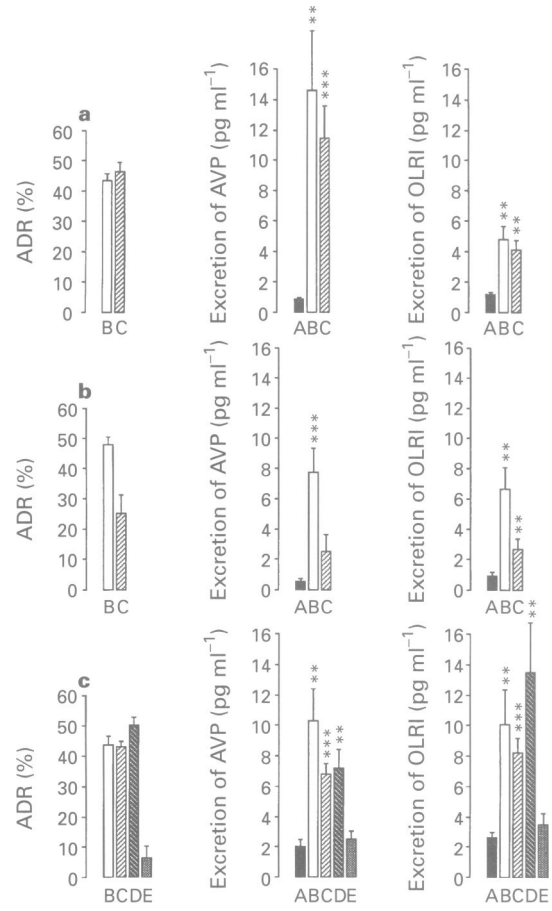

Figure 4 Effect of CNQX and D-AP5 on the ADR and increased urinary excretion of AVP and OLRI produced by muscarine in water-loaded rats under ethanol anaesthesia. In the first series of experiments (a: $n=5$) 2 injections of muscarine 0.1–0.2 nmol were given, the second (C) 60 min after the first (B), to test for tachyphylaxis. In the second series (b: $n=10$) a control response (B) to muscarine (0.05–0.1 nmol) was obtained: CNQX 40 nmol was injected i.c.v. 10 min and 5 min before a second injection (C). In the third series (c: $n=6$) control responses were obtained in the same rat to muscarine (B): 0.1 nmol and NMDA 1–4 nmol (C); D-AP5 4 nmol was injected i.c.v. 10 min and 5 min before a second injection of AMPA (D) and NMDA (E). In each rat a control sample of urine (A) was collected at the start of the experiment. The asterisks above the columns indicate a significant difference in the rate of excretion compared with that in the control sample (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

four experiments: a small residual ADR occurred in two experiments: the rates of excretion of AVP and OLRI were not significantly different from the control values: there were no significant differences between the ADRs and the excretion of AVP and OLRI in response to muscarine before and after D-AP5. These experiments show that the inhibition of the response to muscarine by CNQX can be attributed to an action on AMPA and not NMDA receptors.

The effect of CNQX and D-AP5 on the ADR to HS was investigated. Data were not obtained for the excretion of AVP and OLRI. It was established in previous work (Bisset *et al.*, 1992) that HS does not induce tachyphylaxis on repeated injections. In each experiment two injections of HS were given, one as a control and the other after CNQX or D-AP5. In eight experiments with CNQX, one of which is illustrated in Figure 3b, the mean ADR to the first control injection was $44\pm2.8\%$; in seven experiments the ADR was abolished after CNQX and, in one, it was reduced from 40%–34%. In seven experiments, in which HS and NMDA were tested in the same rat, the ADR to NMDA was abolished in every experiment, but there was no significant difference between the mean response to HS of $40\pm2.12\%$ before and $31\pm6.4\%$ after D-AP5.

Discussion

Glutamate, AMPA and NMDA produced an ADR which was abolished by a AVP antagonist. AMPA, which was about five times more potent than NMDA and one thousand times more potent than glutamate, was effective in a dose as low as 100 pmol i.c.v. For AMPA and NMDA it was shown that the ADR was accompanied by increased urinary excretion of both AVP and OLRI. The EAA antagonist D-AP5 was selective for NMDA; CNQX blocked both AMPA and NMDA. Although the dose of CNQX used was the minimum for effective block of AMPA, it could have blocked NMDA by an action on the glycine site of the NMDA receptor (Lester *et al.*, 1989).

Muscarine was as potent as AMPA; a dose of 50 pmol i.c.v. was effective in producing an ADR and increased excretion of AVP and OLRI. The response to muscarine was significantly inhibited by CNQX but not by D-AP5. This suggests that the release of AVP and oxytocin by muscarine is mediated at least in part by the activation of a glutaminergic input to the SON or PVN involving AMPA receptors. This raises the question of cholinergic transmission in the neural control of release of AVP and oxytocin (Bisset & Chowdrey, 1988). Early work (Hatton *et al.*, 1983a, b) showed that electrical stimulation of the region dorsolateral to the SON in hypothalamic slices increased electrical activity recorded intracellularly from AVP-secreting MNCs in the SON. This effect, which was mimicked by local application of acetylcholine and blocked by hexamethonium, was considered to be due to direct synaptic activation of the MNCs involving a nicotinic cholinoceptor (Cobbett *et al.*, 1986). Cholinergic neurones were detected histochemically in the dorsolateral region which were probably derived from the lateral preoptic nucleus (LPN) (Mason *et al.*, 1983; see also Bisset & Chowdrey, 1988). However, further work failed to reveal synaptic contact between the cholinergic nerve terminals and the dendrites of MNCs in the SON (Theodosis & Mason, 1988; Meeker *et al.*, 1988) and binding sites for nicotine were found to be located outside the SON (Sharp *et al.*, 1987). Gribkoff and his co-workers found that, although local application of nicotine and acetylcholine in hypothalamic slices stimulated MNCs (Gribkoff *et al.*, 1988), their response to electrical stimulation in the dorsolateral region was not blocked by tubocurarine or hexamethonium (Gribkoff & Dudek, 1990). The response was blocked by EAA antagonists (Gribkoff & Dudek, 1990; Gribkoff, 1991). Recently it has been shown that oxotremorine, an agonist acting selectively on muscarinic cholinoceptors, induces an ADR on microinjection into the SON or PVN (Mori *et al.*, 1984) or release of AVP into the circulation on injection into the PVN but not the SON; nicotine caused release on injection into the SON (Shoji *et al.*, 1989; Ota *et al.*, 1992). However, muscarine does not appear to act directly on the MNCs since the binding sites for [^{3}H]-quinuclidinylbenzoate, a selective probe for muscarinic cholinoceptors, are located outside the SON and PVN. Significantly, these binding sites are closely correlated with the axon terminals of cholinergic neurones in the dorsolateral region (Michels *et al.*, 1986). This suggests that the glutaminergic input to the SON is cholinoceptive and that the cholinoceptors are of the muscarinic type. It is possible that the glutaminergic neurones receive a cholinergic innervation from neurones in the LPN which are also cholinoceptive but express nicotinic cholinoceptors. These neurones would be comparable with postganglionic parasympathetic neurones in the autonomic nervous system. Nicotine could act by stimulating the LPN. Electrical stimulation in the dorsolateral region might involve either the cholinergic or the glutaminergic neurones; this might explain the contradictory results obtained by different workers.

The ADR to noradrenaline was not blocked by CNQX. The contrasting responses of muscarine and noradrenaline to

CNQX are consistent with differences in the anatomy of the cholinergic and noradrenergic innervation of the SON and PVN. The noradrenergic input is derived mainly from the A1 group of neurones in the ventrolateral medulla (Sawchenko & Swanson, 1981). Although some neurones appear to terminate in the perinuclear zone (Raby & Renaud, 1989), the innervation is predominantly direct with a close synaptic contact between the noradrenergic axon terminals and the MNCs (Ochiai *et al.*, 1988; Shioda & Nakai, 1992). Noradrenaline should therefore stimulate adrenoceptors on the MNCs (Randle *et al.*, 1985; 1986) without the intervention of an interneurone.

The location of osmoreceptors and the site of action of HS injected i.c.v. are controversial (see Bisset & Chowdrey, 1988). The SON is itself osmosensitive but forms part of an osmoreceptor complex with the circumventricular organs (CVOs) (Dyball & Leng 1989; Honda *et al.*, 1990). Electrical or osmotic stimulation of the organum vasculosum laminae terminalis (OVLT) in hypothalamic slices evokes excitatory postsynaptic potentials in MNCs which are blocked by CNQX and AP5 (Yang *et al.*, 1994; Bourque *et al.*, 1995) and kynurenic acid blocks the release of AVP from hypothalamic slices exposed to increased osmolality (Sladek *et al.*, 1989). In the present experiments, the ADR to hypertonic saline was blocked by CNQX but not NMDA. Data were not obtained for the urinary excretion of AVP and oxytocin. However, in this work ADRs were generally in good agreement with rates of excretion of AVP and, in previous work, an excellent correlation was demonstrated between the ADR and the excretion of AVP in response to hypertonic saline i.c.v. (Bisset *et al.*, 1990). Our results *in vivo* therefore reinforce the evidence from *in vitro* work that release of AVP by hypertonic saline is mediated at least in part by activation of a glutaminergic input to the SON and PVN. The osmoreceptor complex appears to function independently of the cholinergic systems since we have found that the release of AVP and oxytocin by hypertonic saline i.c.v. is not blocked by atropine, hexamethonium or neosurugatoxin (Bisset *et al.*, 1988; 1992).

Our results demonstrate a potent action of AMPA and NMDA in releasing both AVP and oxytocin *in vivo*. This adds support to the evidence for a putative role of endogenous EAAs in the neural control of release of these hormones. We have shown also that muscarine and hypertonic saline cause release not by a direct action on the SON and PVN but by activation of a glutaminergic input involving AMPA receptors which may function as a terminal interneurone in afferent neural pathways to these nuclei.

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References

ANDERSON, W.A., BRUNI, J.E. & KAUFMANN, A. (1990). Afferent connections of the rat's supraoptic nucleus. *Brain Res.*, **24**, 191–200.

BISSET, G.W. & CHOWDREY, H.S. (1988). Control of release of by neuroendrocine reflexes. *J. Exp. Physiol.*, **73**, 811–872.

BISSET, G.W., CHOWDREY, H.S., FAIRHALL, K.M. & GUNN, L.K. (1990). Central inhibition by γ-aminobutyric acid and muscimol of the release of vasopressin and oxytocin by an osmotic stimulus in the rat. *Br. J. Pharmacol.*, **99**, 529–535.

BISSET, G.W., FAIRHALL, K.M. & TSUJI, K. (1992). The effect of neosurugatoxin on the release of neurohypophysial hormones by nicotine, hypotension and an osmotic stimulus in the rat. *Br. J. Pharmacol.*, **106**, 685–692.

BOURQUE, C.W., HU, B., RICHARD, D. & KIRKPATRICK, K. (1995). Exitatory amino acid regulation of rat hypothalamic magnocellular neurosecretory cells (MNCs) *in vivo*. *J. Physiol.*, **483**, 9–10S.

COBBETT, P.J.R., MASON, W.T. & POULAIN, D.A. (1986). Intracellular analysis of control of rat supraoptic neurone (SON) activity *in vitro* by acetylcholine (ACh). *J. Physiol.*, **37**, 216P.

COSTA, A., YASIN, S.A., HUCKS, D., FORSLING, M.L., BESSER, G.M. & GROSSMAN, A. (1992). Differential effects of neuroexcitatory amino acids on corticotropin-releasing hormone-41 and vasopressin release from rat hypothalamic explants. *Endocrinology*, **131**, 2595–2602.

DYBALL, R.E.J. & LENG, G. (1989). Hypothalamic microcircuits involved in osmoregulation. *Biomed. Res.*, **10**, Supplement 3, 21–32.

GRIBKOFF, V.K. (1991). Electrophysiological evidence for N-methyl-D-aspartate excitatory amino acid receptors in the rat supraoptic nucleus *in vitro*. *Neurosci. Lett.*, **131**, 260–262.

GRIBKOFF, V.K., CHRISTIAN, E.P., ROBINSON, J.H., DEADWYLER, S.A. & DUDEK, F.E. (1988). Cholinergic excitation of supraoptic neurones in hypothalamic slices of rat. *Neuropharmacology*, **27**, 721–727.

GRIBKOFF, V.K. & DUDEK, F.E. (1990). Effects of excitatory amino acid antagonists on synaptic responses of supraoptic neurones in slices of rat hypothalamus. *J. Neurophysiol.*, **63**, 60–71.

HATTON, G.I., HO, Y.W. & MASON, W.T. (1983a). Synaptic mediation of supraoptic neuronal activity in slices of rat hypothalamus. *J. Physiol.*, **340**, 41–42P.

HATTON, G.I., HO, Y.W. & MASON, W.T. (1983b). Synaptic activation of phasic bursting in rat supraoptic nucleus neurones recorded in hypothalamic slices. *J. Physiol.*, **345**, 297–317.

HONDA, K., NEGORO, H., DYBALL, R.E.J., HIGUCHI, T. & TAKANO, S. (1990). The osmoreceptor complex in the rat: evidence for interactions between the supraoptic and other diencephalic nuclei. *J. Physiol.*, **431**, 225–241.

LESTER, R.A.J., QUARUM, M.L., PARKER, J.D., WEBER, E. & JAHR, C.E. (1989). Interaction of 6-cyano-7-nitroquinoxaline-2,3-dione with the N-methyl-D-aspartate receptor-associated KGlycine site. *Mol. Pharmacol.*, **35**, 565–570.

MASON, W.T., HO, Y.W., ECKENSTEIN, F. & HATTON, G.I. (1983). Mapping of cholinergic neurones associated with rat supraoptic nucleus: combined immunocytochemical and histochemical identification. *Brain Res.*, **11**, 617–626.

MEEKER, R.B., GREENWOOD, R.S. & HAYWARD, J.N. (1993). Glutamate is the major excitatory transmitter in the supraoptic nuclei. *Ann. N.Y. Acad. Sci.*, **689**, 636–639.

MEEKER, R.B., SWANSON, D.J. & HAYWARD, J.N. (1988). Local synaptic organization of cholinergic neurones in the basolateral hypothalamus. *J. Comp. Neuro.*, **276**, 157–168.

MICHELS, K.M., MEEKER, R.B. & HAYWARD, J.N. (1986). Differential distribution of muscarinic cholinergic and putative nicotinic cholinergic receptors within the hypothalamo-neurohypophysial system of the rat. *Neuroendocrinology*, **44**, 498–507.

MORI, M., TSUSHIMA, H. & MATSUDA, T. (1984). Antidiuretic effects of oxotremorine microinjected into the hypothalamic supraoptic and paraventricular nuclei in a water-loaded and ethanol-anaesthetized rat. *Jpn. J. Pharmacol.*, **35**, 27–36.

OCHIAI, H., IWAI, C. & NAKAI, Y. (1988). Ultrastructural demonstration of the catecholaminergic innervation of vasopressin neurones in the paraventricular nucleus of the rat by double-labelling immunocytochemistry. *Neurosci. Lett.*, **85**, 14–18.

OTA, M., CROFTON, J.T., TOBA, K. & SHARE, L. (1992). Effect on vasopressin release of microinjection of cholinergic agonists into the rat supraoptic nucleus. *Proc. Soc. Exp. Biol. Med.*, **201**, 208–214.

RABY, W.N. & RENAUD, L.P. (1989). Dorsomedial medulla stimulation activates rat supraoptic oxytocin and vasopressin neurones through different pathways. *J. Physiol.*, **417**, 279–294.

RANDLE, J.C.R., BOURQUE, C.W. & RENAUD, L.P. (1985). α_1 Adrenergic receptor activation depolarizes rat supraoptic neurosecretory neurones *in vitro*. *Am. J. Physiol.*, **251**, R569–574.

RANDLE, J.C.R., MAZUREK, K., KNESFEL, D., DUFRESNE, J. & RENAUD, L.P. (1986). α_1 Adrenergic receptor activation releases vasopressin and oxytocin from perfused rat hypothalamic explants. *Neurosci. Lett.*, **65,** 219–223.

RENAUD, L.P., CUNNINGHAM, J.T., NISSEN, R. & YANG, C.R. (1993). Electrophysiology of central pathways controlling release of neurohypophysial hormones. Focus on the lamina terminalis and diagonal band inputs to the supraoptic nucleus. *Ann. of N.Y. Acad. Sci.*, **689,** 122–132.

SAWCHENKO, P.E. & SWANSON, L.W. (1981). The A1 catecholamine cell group: a major source of aminergic input to the paraventricular and supraoptic nuclei in the rat. *Anat. Rec.*, **199,** 225–226A.

SAWYER, W.H., PANG, P.K.T., SETO, J., MCENROE, M., LAMMEK, B. & MANNING, M. (1981). Vasopressin analogues that antagonise antidiuretic responses by rat to the antidiuretic hormone. *Science*, **212,** 49–51.

SHARP, B.M., NICOL, S., CUMMINGS, S. & SEYBOLD, V. (1987). Distribution of nicotinic binding sites with respect to CRF and neurophysin immunoreactive perikarya within the rat hypothalamus. *Brain Res.*, **422,** 361–366.

SHIODA, S. & NAKAI, Y. (1992). Noradrenergic innervation of vasopressin-containing neurones in the rat hypothalamic supraoptic nucleus. *Neurosci. Lett.*, **140,** 215–218.

SHOJI, M., SHARE, L., CROFTON, J.T. & BROOKS, D.P. (1989). The effect on vasopressin release of microinjection of cholinergic agonists into the paraventricular nucleus of conscious rats. *J. Neuroendocrinol.*, **1,** 401–406.

SLADEK, C.D., GALLAGHER, M. & YAGIL, C. (1989). Evidence for involvement of excitatory amino acids in osmotic stimulation of vasopressin release. *Soc. Neurosci. Abstracts*, **15,** 1077.

THEODOSIS, D.T. & MASON, W.T. (1988). Choline acetyltransferase immunocytochemical staining of the rat supraoptic nucleus and its surroundings. *Cell Tissue Res.*, **254,** 119–124.

TRIBOLLET, E., ARMSTRONG, W.E., DUBOIS-DAUPHIN, M. & DREIFUSS, J.J. (1985). Extra-hypothalamic afferent inputs to the supraoptic nucleus area of the rat as determined by retrograde and anterograde tracing techniques. *Neuroscience*, **15,** 135–148.

VAN DEN POL, A.N., WUARIN, J.-P. & DUDEK, F.E. (1990). Glutamate, the dominant excitatory transmitter in neuroendocrine regulation. *Science*, **250,** 1276–1278.

YANG, C.R., SENATOROV, V.V. & RENAUD, L.P. (1994). Organum vasculosum lamina terminalis-evoked postsynaptic responses in rat supraoptic neurones *in vitro*. *J. Physiol.*, **477,** 59–74.

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