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Central inhibition by γ-aminobutyric acid of the release of vasopressin by carbachol in the rat

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1 γ-Aminobutyric acid (GABA) inhibited the antidiuretic response and the increased urinary excretion of vasopressin produced by carbachol when both drugs were injected into a lateral cerebral ventricle (i.c.v.) in the water-loaded rat under ethanol anaesthesia.

2 The inhibitory effect of GABA was mimicked by muscimol and 3-amino-1-propane sulphonic acid (3-APS) and blocked by bicuculline.

3 GABA injected i.v. or into the cisterna magna (i.cist.) did not inhibit the release of vasopressin by carbachol injected i.c.v.

4 The results suggest a role for GABA as a putative inhibitory transmitter in the hypothalamo-neurohypophysial system, acting directly on the supraoptic or paraventricular nuclei in the anterior hypothalamus.

Introduction

In confirmation of earlier work by Kühn & McCann (1970, 1971) and Kühn (1974) it was found that carbachol injected into a lateral cerebal ventricle in the water-loaded rat under ethanol anaesthesia produced an antidiuretic response accompanied by increased excretion of vasopressin in the urine (Bisset & Chowdrey 1980; 1981; 1984). This response did not occur in the Brattleboro rat with congenital diabetes insipidus and was attributed to release of vasopressin from the neurohypophysis. A drug injected into a lateral ventricle may pass into the third and fourth ventricles and then through the lateral recesses of the fourth ventricle into the subarachnoid space from which the dorsal and ventral surfaces of the brainstem are accessible. However, there is no terminal aperture (foramen of Magendie) in the fourth ventricle of the rat (see Feldberg, 1976) and a drug injected into the cisterna magna cannot enter the ventricles. Carbachol was less than one hundred times as effective when injected into the cisterna (i.cist.) as into the lateral ventricle (i.c.v.). This suggests that carbachol releases vasopressin by acting on structures reached from the walls of the ventricles. Possible sites of action are the supraoptic and paraventricular nuclei (SON and PVN) in the anterior hypothalamus from which vasopressinergic neurones project to the neurohypophysis.

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In this paper it is shown that the release of vasopressin by carbachol can be inhibited by γ-aminobutyric acid (GABA) injected i.c.v., but not i.cist. or i.v. This implies a role for GABA as a putative inhibitory neurotransmitter acting centrally on the hypothalamo-neurohypophysial system. A preliminary account of this work has been published (Bisset & Chowdrey, 1980).

Methods

The experiments were carried out on male CSE Wistar rats weighing approximately 250 g. A diuresis was established under ethanol anaesthesia by maintaining a constant fluid load equivalent to 8% of the body weight. Cannulae were inserted into a lateral cerebral ventricle and the cisterna magna. Blood pressure was recorded from a femoral artery and urine flow with a drop counter set to a 1 min time base. For the experiments in Table 1, 5 ml samples of urine were collected during control periods and immediately after i.c.v. injections of carbachol and muscarine or i.v. injections of vasopressin. The time required to excrete the 5 ml samples was noted and the mean rate of urine flow calculated. The percentage reduction in rate produced by drug injections was taken as a measure of the antidiuretic response. Vasopressin was extracted

from the urine samples and estimated by bioassay of antidiuretic activity. The recovery of vasopressin added to control samples of urine in the seven experiments of Table 1 varied from 86–100% and the value in the table have been corrected for any loss of activity on extraction. Intravenous injections were made through a cannula in the external jugular vein. The volume of drug solutions injected i.c.v. or i.cist. did not exceed 4 μl. Full details of the experimental procedures have been published (Bisset & Chowdrey, 1984).

Drugs

Drugs used were: carbachol chloride (carbachol injection, B.P., Evans Medical), vasopressin (Pitressin, Parke Davis), GABA (BDH), muscarine chloride, 3-amino-1-propane sulphonic acid (3-APS), muscimol and bicuculline methobromide (Sigma). Doses of carbachol, muscarine and bicuculline are expressed in terms of their salts.

Results

A typical antidiuretic response to carbachol and its inhibition by GABA, when both drugs are injected i.c.v., is illustrated in Figure 1. The injection of 40 ng carbachol i.c.v. produced an antidiuretic response similar in profile, amplitude and duration to that produced by i.v. injection of 0.5 mu vasopressin, but with a slightly delayed onset. Carbachol also caused a rise of blood pressure of about 10 mmHg which was larger and of longer duration than the small pressor response to vasopressin. A smaller dose of carbachol, 20 ng, produced a brief pressor and a diuretic, but no antidiuretic response. The injection of 40 μg GABA i.c.v. had a negligible effect on blood pressure and urine flow but completely inhibited the antidiuretic response to three successive doses of 40 ng carbachol (1, 2 and 3) injected i.c.v., 1, 7 and 13 min after GABA. Figure 1 shows that the pressor response to the first of the three injections of carbachol was also abolished by GABA. A fourth injection of 40 ng carbachol i.c.v. (4) 19 min after GABA produced almost identical antidiuretic and pressor responses to those obtained before GABA. This shows that the inhibitory effect of GABA is brief and reversible. An inhibitory effect of GABA in doses of 4–160 μg, i.c.v. on the antidiuretic and pressor responses to 10–50 ng carbachol i.c.v. was observed consistently in 22 experiments.

The contrasting effects of GABA injected i.c.v. and i.cist. are illustrated in Figure 2. Figure 2a shows two control responses to 20 ng carbachol i.c.v. The second

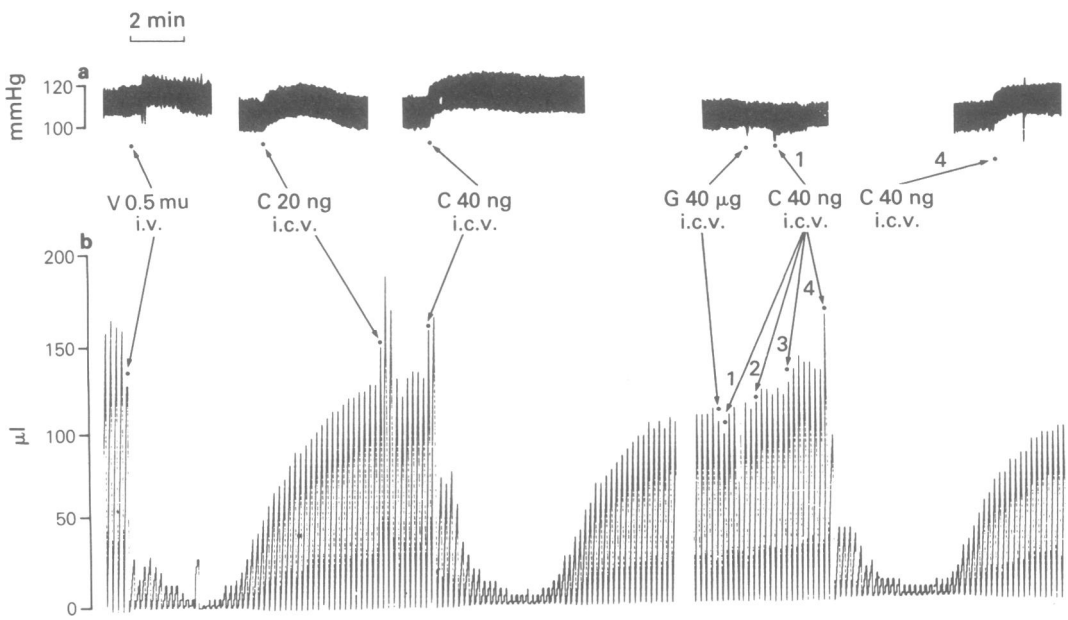

Figure 1 Blood pressure (a) and urine flow (b) in a water-loaded rat under ethanol anaesthesia with a constant fluid load equivalent to 8% of the body weight. Each vertical line in the trace of urine flow indicates the volume (μl) excreted in 1 min. Note the different time scale for recording of blood pressure. Injections were given intravenously (i.v.) or into a lateral cerebral ventricle (i.c.v.). V = vasopressin; C = carbachol; G = GABA.

dose was injected before full recovery from the first dose and the similarity of the responses shows that no tachyphylaxis occurs. As in the experiment of Figure 1, the response to carbachol was reversibly inhibited by a small preceding dose (20 μg) of GABA. In Figure 2b an injection of 100 μg GABA i.cist. is shown to produce a slight antidiuretic and depressor response. A further dose of 20 ng carbachol i.c.v. 2 min after a

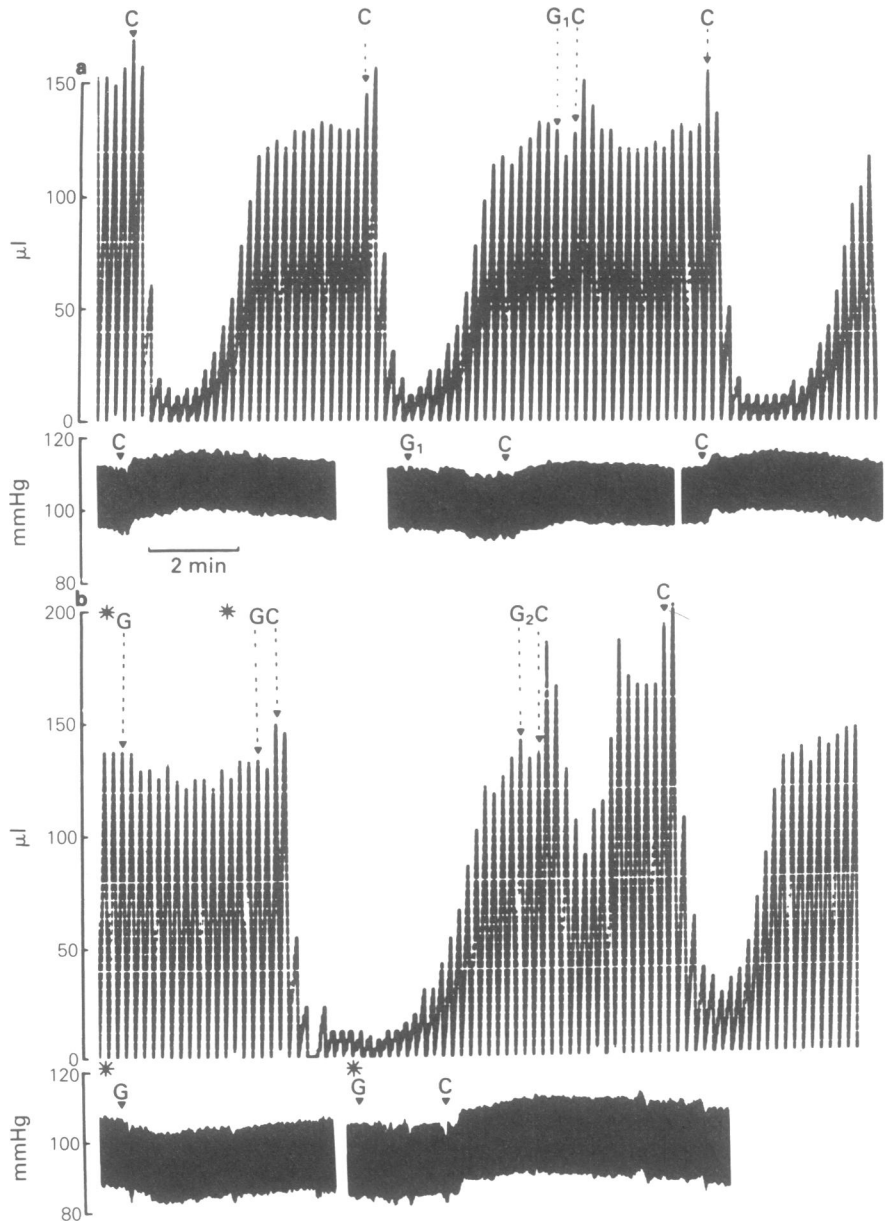

Figure 2 Blood pressure and urine flow in a water-loaded rat recorded as in legend to Figure 1. C = carbachol 20 ng i.c.v.; G = GABA: G_1 20 μg, G_2 10 μg i.c.v.; G* 100 μg injected into the cisterna magna (i.cist.).

second injection of 100 μg GABA i.cist. produced a larger antidiuretic response than in the original controls, although a final injection of only 10 μg GABA i.c.v., one half the dose shown in Figure 2a, produced a reversible inhibition of the response to 20 ng carbachol i.c.v. This shows that GABA is at least 10 times more effective i.c.v. than i.cist. in inhibiting the antidiuretic response to carbachol. Similar results were obtained in four other experiments in one of which a dose of only 5 μg GABA i.c.v. was sufficient to block the response to carbachol 5 ng but a dose of 320 μg GABA i.cist. was ineffective.

GABA injected i.v. did not inhibit the antidiuretic response to i.c.v. carbachol. This is illustrated in Figure 3. A large antidiuretic response was obtained to 10 ng carbachol i.c.v. The injection of 100 μg GABA i.v. caused a fall of blood pressure of about 30 mmHg and a small antidiuretic response, probably due to reflex release of vasopressin. Increasing doses up to 4 mg caused small falls of blood pressure and only slight reductions in urine flow. A second dose of carbachol, 10 ng, injected i.c.v. 2 min after the injection of 4 mg GABA i.v. produced as large an antidiuretic response as the first dose before GABA, although the response to a third injection of 10 ng carbachol was abolished by two preceding injections of only 40 μg GABA i.c.v. In three other experiments in which GABA 5 μg, i.c.v. inhibited the response to carbachol 5–20 ng i.c.v. doses of 0.5–4 mg GABA i.v. were ineffective.

The inhibitory effect of GABA i.c.v. on the antidiuretic response to carbachol was blocked in five experiments when it was administered either with an equal dose of bicuculline or after i.v. infusion of 250 μg bicuculline. In four experiments this inhibitory effect was mimicked by 3-amino-1-propane-sulphonic acid

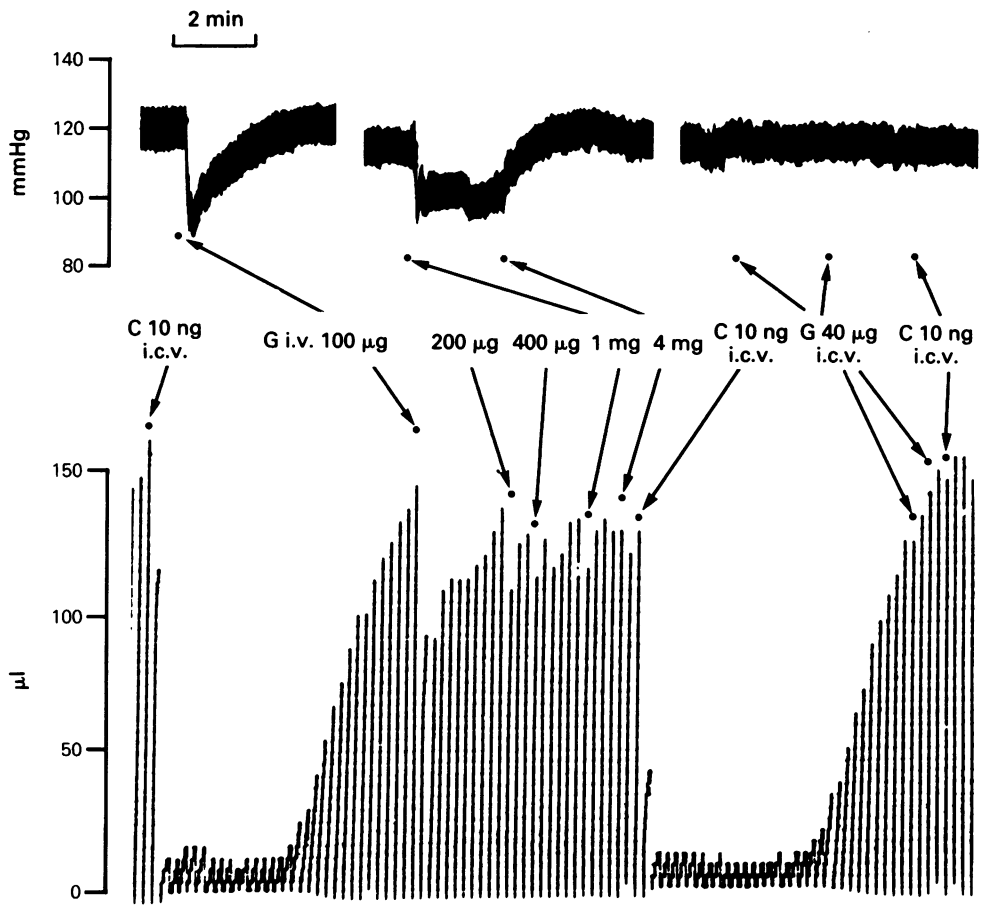

Figure 3 Blood pressure and urine flow in a water-loaded rat recorded as in legend to Figure 1. C = carbachol; G = GABA, injected i.v. or i.c.v.

(3-APS) and in one by muscimol. In doses of 20–100 μg 3-APS produced a much longer lasting inhibition of the antidiuretic response to carbachol than that obtained with GABA. In contrast with GABA and 3-APS, as little as 130 ng muscimol i.c.v. was found to be sufficient to abolish the antidiuretic response to 10 ng carbachol i.c.v.

The results of seven experiments in which the urinary excretion of vasopressin was measured in 5 ml samples of urine, are given in Table 1. The volume of 5 ml was found to be sufficient to ensure that, during an antidiuretic response, the urine flow had recovered completely or almost completely before the end of the collection. It could then be assumed that release of vasopressin into the circulation had ceased and full recovery of the fraction excreted in the urine was obtained. In three experiments, 5, 6 and 7, the amounts of vasopressin recovered from the urine after i.v. injections of 1–2 mu vasopressin were 12, 7 and 12% of the injected dose. Vasopressin was detected in the initial control samples in three experiments (1, 4 and 5) in amounts of 17, 19 and 24 μu despite the fact that the rate of urine flow increased during the collection of the samples; in the other four experiments, none was detected (< 8 to < 35 μu). In all experiments the first injections of carbachol 40–80 ng or muscarine 40 ng

Table 1 Excretion of vasopressin in 5 ml samples of urine collected during a control period and during the antidiuretic response to i.c.v. or i.v. injections of drugs

		Mean rate of urine flow (μl min^{-1})			
Expt	*Drug*	*Before collection*	*During collection*	*Change* (%)	*Vasopressin* (μu) *in 5 ml urine*
1	Control	115	122	+ 6	17
	C 40 ng	100	89	− 11	66
	GABA 40 μg + C 40 ng	151	185	+ 23	17
2	Control	128	161	+ 21	< 8
	C 50 ng	175	89	− 49	105
	GABA 80 μg + C 50 ng	150	106	− 29	23
	C 50 ng	150	83	− 43	109
3	Control	132	185	+ 40	< 20
	C 80 ng	225	116	− 48	240
	GABA 100 μg + C 80 ng	174	139	− 20	29
	C 80 ng	167	98	− 41	219
4	Control	117	132	+ 13	19
	C 40 ng	162	78	− 52	347
	GABA 20 μg + C 40 ng	184	104	− 44	82
	GABA 40 μg + C 40 ng	212	135	− 36	56
	C 40 ng	201	89	− 56	121
5	Control	133	162	+ 24	24
	C 40 ng	173	83	− 52	415
	GABA 100 μg + C 40 ng	152	142	− 7	20
	C 40 ng	201	119	− 41	268
	V 2 mu i.v.	170	98	− 42	245
6	Control	201	208	+ 4	< 20
	C 50 ng	201	104	− 48	348
	GABA 40 μg + C 50 ng	166	114	− 31	111
	GABA 100 μg + C 50 ng	201	147	− 27	19
	V 2 mu i.v.	239	128	− 46	149
7	Control	151	205	+ 36	< 35
	M 40 ng	238	132	− 45	199
	GABA 50 ug + M 40 ng	190	161	− 15	< 38
	M 40 ng	151	86	− 43	218
	V 1mu i.v.	186	102	− 45	120

GABA injected 1–2 min before carbachol (C) or muscarine (M); V = vasopressin.

produced an antidiuretic response with 11 to 52% reduction in the rate of urine flow and an increase in the vasopressin excreted in the urine to amounts varying from 66 to 415 μu. In every experiment the injection of 40–100 μg GABA 1–2 min before carbachol or muscarine inhibited the antidiuresis and reduced the amount of vasopressin excreted in the urine to levels comparable with those observed in the initial controls. In experiments 2 and 7 a second injection of carbachol or muscarine following GABA produced practically the same degree of antidiuresis and urinary excretion of vasopressin as after the first injection; in experiments 3, 4 and 5 an incomplete, but substantial recovery was obtained. Experiments 4 and 6 illustrate graded inhibitory effects of two doses of GABA, 20 and 40 and 40 and 100 μg respectively.

Discussion

It was shown previously that carbachol causes release of vasopressin when injected i.c.v. in the rat, but has little or no effect i.v. or i.cist. (Bisset & Chowdrey, 1984). The present experiments show that GABA blocks both the antidiuretic response and the increased urinary excretion of vasopressin produced by carbachol i.c.v. when GABA itself is injected by this route, but not i.v. or i.cist. Two analogues of GABA were effective i.c.v. in blocking the antidiuretic response to carbachol; muscimol was much more potent than GABA and 3-APS produced a longer duration of action. The action of GABA appeared to be on the A type of receptor (Bowery *et al.*, 1984) since it was blocked by bicuculline.

The exact sites of action of carbachol and GABA are uncertain. Acting from the cerebral ventricles, carbachol must stimulate either the SON or PVN directly, or an excitatory afferent neural input to these nuclei. Acetylcholine applied by microiontophoresis to vasopressin-secreting cells in the SON of the rat prolongs the periodic phasic bursts of firing (Bioulac *et al.*, 1978; Arnauld *et al.*, 1983). Acetylcholine and nicotine stimulate release of vasopressin from the organ cultured rat hypothalamo-neurohypophysial system and this release is inhibited by ganglion blocking agents (Sladek & Knigge, 1977; Sladek & Joynt, 1979). Electrical stimulation of cholinergic neurones in the lateral preoptic nucleus or direct application of acetylcholine by microperfusion in hypothalamic slices from the rat enhance the firing of vasopressin-secreting neurones and this effect is blocked by hexamethonium (Hatton *et al.*, 1983; Cobbett *et al.*, 1986). These experiments indicate that the cholinoceptor on the vasopressin-secreting cells is nicotinic. However, muscarine is equipotent with carbachol in releasing vasopressin on i.c.v. injection in the rat and the release by carbachol is blocked by atropine, but not by hexamethonium (Kühn 1974; Bisset & Chowdrey, 1984). This suggests that carbachol acts on a muscarinic receptor to release vasopressin and that it acts not directly on the SON or PVN, but by stimulating an afferent neural input. In the cat, nicotine also releases vasopressin by stimulating an afferent pathway to the SON and PVN. This arises from a circumscribed zone, the 'nicotine-sensitive zone' (NSZ) on the ventral surface of the medulla (Bisset *et al.*, 1975). However, if carbachol acted at a similar site in the rat it would be effective on i.cist. injection. The nucleus of the tractus solitarius (NTS) which received afferents from peripheral receptors in the cardiovascular system controlling the release of vasopressin in response to changes in blood volume or pressure ('volume control'), and which projects to the SON, is another possible site of action of carbachol, but again this would be accessible to i.cist. injections. The circumventricular organs which have been implicated in osmotic control of vasopressin release also project to the SON and PVN, but since they lie outside the blood-brain barrier, they would be equally accessible to i.v. injection and carbachol is ineffective by this route. A likely site of action of carbachol, therefore, is in the region of the anterior hypothalamus where it may stimulate an afferent neural input to the SON or PVN.

There are two sites at which GABA has already been demonstrated to have an action in inhibiting the release of vasopressin. The first is a presynaptic site at nerve terminals in the neural lobe. Nerve endings immunoreactive to the GABA precursor, glutamic acid decarboxylase (GAD) impinge upon these terminals (Tappaz *et al.*, 1982). Application of GABA inhibits the release of oxytocin or vasopressin assayed by milk-ejecting activity (Dyball & Shaw, 1979) and depresses the antidromically conducted compound action potential (CAP) recorded from the region of the median eminence (Zingg *et al.*, 1979) in response to electrical stimulation of the isolated neural lobe of the rat. The second site of action of GABA is on the ventral surface of the medulla. Topical application of GABA to the NSZ in the cat blocks the release of vasopressin in response to carotid occlusion and application of GABA antagonists stimulates the release of vasopressin (Feldberg & Rocha e Silva, 1978; 1981). This led to the suggestion that the NSZ might act as a relay station for an afferent excitatory projection from the NTS to the SON and PVN and that it is under tonic inhibition by a GABAergic pathway (see also Bisset & Chowdrey, 1984). An afferent projection from the NTS to the NSZ has been demonstrated although the neurotransmitter was not identified (Errington & Dashwood, 1979). In the rabbit the injection of bicuculline into the region of the ventro-lateral medulla which contains the A1 group of noradrenergic neurones selectively innervating the vasopressin-secreting neurones in the SON and PVN

stimulates release of vasopressin (Sved *et al.*, 1982). This suggests that the noradrenergic input is excitatory to the SON and PVN and inhibited by GABA. GAD immuno-reactive neurones have been demonstrated in the NTS (Blessing *et al.*, 1984) which might receive the primary afferents from cardiovascular receptors and project to the Al group or to the NSZ, these two structures possibly being identical. These two sites of action can be excluded in the present experiments for two reasons. First, the neural lobe lies outside the blood-brain barrier and the NTS and ventral surface of the medulla are accessible from the cisterna magna and GABA was ineffective in inhibiting release of vasopressin by carbachol when injected i.v. or i.cist. Secondly, it has been shown that carbachol itself does not act at either of these sites.

These experiments, therefore, suggest a third site of action of GABA in the anterior hypothalamus. Relatively high levels of GABA have been detected in the SON and PVN of the rat (Van der Heyden *et al.*, 1979) and GAD immunoreactive endings in and around the nuclei (Tappaz *et al.*, 1982). GABA may thus inhibit the release of vasopressin by a direct action on the SON or PVN. This could block the effect of stimulation of an excitatory afferent input by carbachol. In view of the brief duration of action of GABA, and the greater distance over which it would have to diffuse from the ventricles to reach the SON, the PVN is the more likely site of action.

The question arises whether endogenous GABA has a physiological role in regulating the release of vasopressin. GAD immunoreactivity in the PVN is not reduced by lesions of afferent pathways to the PVN from the hippocampus and other structures in the CNS (Tappaz & Brownstein, 1977). This suggests that GABAergic fibres arise from short interneurones within the anterior hypothalamus. Endogenous GABA from this source could be released at the final synapse on afferent pathways to the SON and PVN subserving both osmotic and volume control of vasopressin release. GABA i.c.v. but not i.cist., blocks the release of vasopressin by hypertonic saline i.c.v. under the same experimental conditions as in the present paper (Bisset and Chowdrey, unpublished). It has also been shown to inhibit the pressor response (Brennan *et al.*, 1984; Haywood & Brennan, 1985) and the increased plasma concentration of vasopressin (Iovino *et al.*, 1983) produced by hypertonic saline in the rat. An inhibitory effect of GABA on the release of vasopressin by hypovolaemia has also been reported (Knepel *et al.*, 1980).

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