Br. J. Pharmac. (1977), **60**, 77–81

CENTRAL MECHANISM OF VASOPRESSIN-INDUCED CHANGES IN ANTIDIURETIC HORMONE RELEASE

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1 Intracerebroventricularly (i.c.v.) administered vasopressin (0.001–1.0 u) in dogs anaesthetized with chloralose produced a dose-dependent increase in urine flow with a concomitant decrease in the levels of antidiuretic hormone (ADH) in jugular vein blood.

2 Higher doses of vasopressin (1.5–2.0 u, i.c.v.) on the other hand had an antidiuretic effect and produced an increase in blood ADH level.

3 Pretreatment (i.c.v.) with a β-adrenoceptor antagonist completely blocked the diuretic response of low doses of vasopressin (i.c.v.) but did not influence the antidiuretic response obtained with high doses.

4 Repeated administration of vasopressin (1.0 u, i.c.v.) induced tachyphylaxis; central catecholamine depletion with tetrabenazine significantly inhibited the vasopressin-induced diuretic response.

5 It is concluded that intracerebroventricular vasopressin-induced changes in ADH secretion are mediated through the release of catecholamines in the central nervous system.

Introduction

In contrast to the well known peripheral antidiuretic effect of vasopressin (antidiuretic hormone, ADH), the administration of vasopressin into the lateral cerebral ventricles produced a diuretic response in normal dogs (Varma, Jaju & Bhargava, 1969) as well as in spinal transected cats (Nashold, Mannarino & Robinson, 1963). Since haemodynamic changes were observed concomitantly and ADH blood level changes were not measured by these workers, it is difficult to attribute the diuretic response following intracerebroventricular injection of vasopressin, to an inhibition of ADH release. The present study was undertaken to investigate the mechanism of the central vasopressin-induced changes in the release of antidiuretic hormone.

Methods

The study was carried out in 38 mongrel dogs of either sex, weighing between 10–15 kg. The animals were fasted for 24 h but were allowed water *ad libitum*. They were anaesthetized with 10 ml/kg of warm 1% α-chloralose in 0.9% w/v NaCl solution (saline) injected intravenously (Yoshida, Ibayashi, Murakawa & Nakao, 1965). To maintain hydration and anaesthesia, a continuous drip of saline containing 0.1% chloralose was delivered through a femoral vein at a rate just exceeding the rate of urine flow. In another set of experiments, in order to elicit the full antidiuretic effect of higher doses of intracerebroventricular (i.c.v.) vasopressin, the urine flow rate was kept at a higher level by the administration of dextrose-saline (Bhargava, Kulshrestha & Srivastava, 1972; Bhargava, Kulshrestha, Santhakumari & Srivastava, 1973).

Blood pressure was recorded on a kymograph from a carotid artery. Both the ureters were cannulated and connected to a photoelectric drop recording assembly to measure the urine flow. One of the jugular veins was cannulated with a polyethylene indwelling cannula directed towards the head end, to collect blood for ADH estimations. The ADH in the blood was extracted on a column of XE-64 resin by the method of Weinstein, Berne & Sachs (1960) as modified by Yoshida, Motohashi, Ibayashi & Okinaka (1963). This method of extraction was found to give a recovery of 80% ADH in controls and results were modified accordingly. The ADH in the eluate was estimated by the antidiuretic assay method in rats (Dicker, 1953). Arginine-vasopressin (Pitressin, Parke-Davis) was used as a standard in the bioassay.

Bilateral vagotomy, sino-aortic denervation, adrenalectomy and spinal cord transection at C2 level were performed by standard techniques in different experiments. Two or three hours after the surgical procedure, when the urine flow rate was constant over a period of 30 min, the drugs were administered through a cannula implanted in one of the lateral cerebral ventricles (Bhargava & Tangri, 1959). The

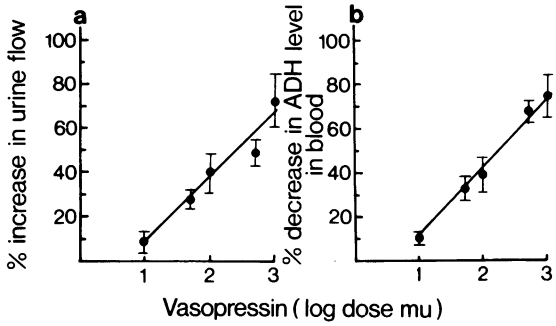

Figure 1 Effect of graded doses (0.001–1.0 u) of vasopressin (i.c.v.) on (a) urine flow and (b) blood ADH concentration in dogs. Vasopressin induced a diuretic response accompanied by a corresponding fall in blood ADH titre.

correct placement of the cannula was confirmed at autopsy. The total volume of fluid injected never exceeded 0.5 ml; in control experiments an equal volume of saline did not influence the urine flow.

Drugs

The following drugs were used: vasopressin (Pitressin, Parke-Davis), *N*-isopropyl-*p*-nitrophenyl ethanolamine hydrochloride (INPEA, Selvi & Co.), (±)-propranolol hydrochloride (ICI), atropine sulphate (E. Merck), phenoxybenzamine hydrochloride (SKF) and tetrabenazine (Roche). The doses of compounds used refer to their salts.

Results

Effect of vasopressin (i.c.v.) on urine flow and blood ADH level

Vasopressin injected (i.c.v.) in graded doses of 0.001–1.0 unit, consistently produced an increase in urine flow that was associated with a corresponding fall in blood ADH concentration (Table 1). The log dose of vasopressin was found to be linearly related to the diuretic response or the fall in blood ADH titre in this dose range (see Figure 1). The diuretic response observed with 1.0 unit of vasopressin started within 10–20 min after injection, the peak effect was observed within 40–60 min and gradual recovery occurred in 90–120 min (Figure 2). With smaller doses (<1.0 unit) of vasopressin (i.c.v.) the diuretic response was of shorter duration depending upon the dose.

Higher doses of vasopressin (1.5–2.0 u, i.c.v.), on the other hand, induced an antidiuretic response (also shown in Figure 2), with a concomitant increase in blood ADH titre. With all the doses of vasopressin (i.c.v.) there was a rise in blood pressure with 1.0 unit of vasopressin, ranging from 5 to 20 mmHg. In all instances, the pressor response never lasted more than 10–20 minutes.

Effect of repeated (i.c.v.) administration of vasopressin

Repeated injections of 1.0 unit vasopressin (i.c.v.) led to gradual decline in the diuretic response and after the third or fourth dose the diuretic response to vasopressin was not obtainable. However, smaller doses (0.001–0.5 u) of vasopressin, on repeated

Table 1 Effect of intracerebroventricular vasopressin on urine flow and antidiuretic hormone (ADH) levels in the jugular vein blood of dog

Vasopressin (units i.c.v.)	*ADH level (u/ml ± s.e. mean) in jugular vein blood at peak effect*		*% Increase in urine flow at peak effect*
	Control	*After vasopressin (i.c.v.)*	
0.01		11.7 ± 0.9	8
0.05		10.2 ± 1.4	28
0.10	15.2 ± 1.3	7.4 ± 0.6	40
0.50	(*n* = 12)	5.4 ± 0.5	50
1.0		5.0 ± 0.6	74
2.0 (Hydrated dogs)	4.2 ± 0.6 (*n* = 5)	10.2 ± 3.6	−34.6 ± 2.8*

* Decrease in urine flow.

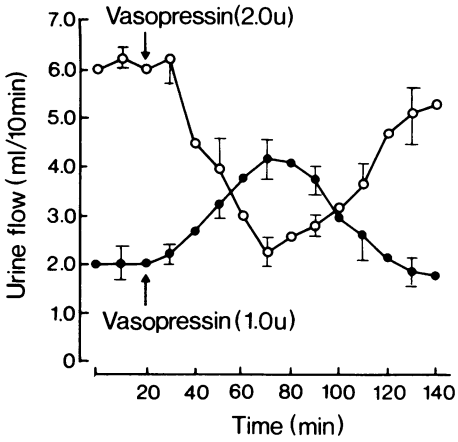

Figure 2 Time-response curve of intracerebroventricular administration of vasopressin (1.0 and 2.0 u)-induced changes in urine flow. Vasopressin (i.c.v.) in a dose of 1.0 unit elicited a diuretic response which began within 10–20 min and reached a peak between 40–60 min; complete recovery occurred in 90–120 minutes. In contrast 2.0 units of vasopressin (i.c.v.), induced an antidiuretic response of a similar time course to that obtained with 1.0 unit.

administration did not produce significant tachyphylaxis.

Effect of surgical procedures on diuretic response to (i.c.v.) vasopressin

Spinal cord transection (C2), bilateral adrenalectomy, bilateral vagotomy and/or sino-aortic denervation did not affect significantly the vasopressin (0.5 u and 2.0 u, i.c.v.)-induced diuretic and antidiuretic responses ($P > 0.05$) respectively. However, the pressor response obtained with vasopressin (i.c.v.) was completely blocked by bilateral adrenalectomy.

Effect of central drug pretreatment on (i.c.v.) vasopressin-induced diuretic response

Prior central atropinization (2.0 mg i.c.v.) did not influence the diuretic response obtained with vasopressin (1.0 u, i.c.v.). Phenoxybenzamine (2.0 mg, i.c.v.), an α-adrenoceptor blocking agent failed to prevent the vasopressin (1.0 u, i.c.v.)-induced increase in urine outflow or the fall in blood ADH concentration. However, the β-adrenoceptor blocking agents, INPEA (10 mg, i.c.v.) or propranolol (2 mg, i.c.v.) given 15 min beforehand, completely blocked the diuretic response to 1.0 unit of vasopressin (i.c.v.). Tetrabenazine (30 mg/kg i.p., given 4 h beforehand), a selective central catecholamine depletor, also blocked the vasopressin-induced response and fall in blood ADH titre ($P < 0.05$).

The antidiuretic response obtainable with higher doses of vasopressin (1.5 and 2.0 u) was not blocked by propranolol. Central α-adrenoceptor blockade by phenoxybenzamine prevented the antidiuretic response to high (i.c.v.) doses (1.5–2.0 u) of vasopressin in 3 out of 5 animals; in the remaining 2 dogs this dose of vasopressin elicited a diuretic response after phenoxybenzamine pretreatment.

Discussion

In this study, intracerebroventricular vasopressin (0.001 to 1.0 u) produced a dose-dependent increase in urine flow in dogs which was associated with a corresponding fall in ADH titre of jugular vein blood (Figure 1, Table 1). Only with higher doses of vasopressin (0.5–1.0 u, i.c.v.) was there also a rise in blood pressure (5–20 mmHg) which returned to a normal level within 20 minutes. The diuretic response of centrally administered vasopressin (1.0 u) started within 10–20 min, the peak effect was observed within 40–60 min and gradual recovery occurred in 90–120 minutes. From the time course of the diuretic response, it appears that haemodynamic factors (BP rise) are not responsible for increase in urine flow. Furthermore, the pressor response to vasopressin (i.c.v.) was not elicited in spinal transected or bilaterally vagotomized animals, whereas, prior spinal transection, bilateral vagotomy, sino-aortic denervation or adrenalectomy did not influence the diuretic response. These observations confirm that the vasopressin-induced diuretic response is of central origin and is mediated through central inhibition of ADH release.

Central receptors concerned in the control of antidiuretic hormone release were characterized by Bhargava *et al.* (1972). By studying the effects of intracerebroventricular administration of various cholinoceptor and adrenoceptor agonists and antagonists, it was concluded that central muscarinic cholinoceptors and α-adrenoceptors are facilitatory and β-adrenoceptors are inhibitory for the release of ADH. In the present study, central atropinization or α-adrenoceptor blockade with phenoxybenzamine (2.0 mg i.c.v.) did not block the vasopressin (i.c.v.)-induced diuretic response. Central pretreatment with INPEA (10 mg) or propranolol (2 mg), on the other hand, completely blocked the vasopressin (i.c.v.)-induced increase in urine flow or decrease in ADH titre of jugular vein blood ($P < 0.05$). These observations suggest that the (i.c.v.) vasopressin-induced diuretic response is mediated through central β-adrenoceptors.

In our experiments, repeated intracerebroventricular administration of vasopressin (1.0 u)

induced tachyphylaxis. This would suggest an indirect central mechanism responsible for the inhibition of ADH release. Failure of vasopressin (i.c.v.) to induce a diuretic response in dogs pretreated with tetrabenazine (30 mg/kg i.p.), a selective central catecholamine depletor, supports an indirect mechanism of action. It is our contention that vasopressin (i.c.v.) releases catecholamines centrally and this is responsible for the activation of a central adrenergic mechanism for the inhibition of ADH release. In this connection, Statt & Chenoweth (1966) have demonstrated more than a 100% increase in catecholamine content of cavernous sinus blood following intravenous injection of vasopressin in dogs.

The antidiuretic response induced by high doses of vasopressin (1.5–2.0 u, i.c.v.) may arise either from a release of ADH or may be the result of peripheral leakage of the centrally administered vasopressin. The antidiuretic response to high doses of vasopressin (1.5–2.0 u, i.c.v.) did not bear any temporal relationship to the pressor response; the pressor response was inconsistent and of shorter duration (<20 min) as compared to the antidiuretic response. The antidiuretic response was associated with a concomitant rise in the ADH concentration in jugular vein blood and it was successfully antagonized by phenoxybenzamine (2.0 mg, i.c.v.). Furthermore, a local vasoconstrictor action of vasopressin cannot be held responsible for the antidiuretic effect. In 2 out of 5 dogs pretreated with phenoxybenzamine, the vasopressin-induced antidiuresis was converted to a diuretic response. It seems that central α-adrenoceptor blockade is adequate to block the antidiuretic response and the subsequent diuretic response may be due to unmasking of a central β-adrenoceptor activation. These findings suggest a central action of vasopressin in eliciting the antidiuretic response as well. It appears that higher doses of vasopressin release larger amounts of catecholamines centrally and thereby predominantly activate the central α-adrenoceptors which must be responsible for the antidiuretic response. This is consistent with the observation that low concentrations of catecholamines selectively activate β-receptors and higher concentrations are required to activate α-receptors (Innes & Nickerson, 1970). In a previous study, intracerebroventricular injection of histamine was shown to induce an antidiuretic response which has been attributed to a massive release of catecholamines (Bhargava *et al.*, 1973). It appears that vasopressin also influences central ADH release through an adrenergic mechanism.

The foregoing results unequivocally demonstrate that the antidiuretic hormone (vasopressin) when introduced into the ventricular system of brain inhibits its own release and thereby elicits a diuretic response, i.e. an inhibitory feed back mechanism for ADH exists. ADH activity in the cerebrospinal fluid has been reported (Hoyle, 1933; Talanti & Kivalo, 1961; Heller & Ginsburg, 1966). However, it would be premature to suggest that this 'feed back' mechanism is operative in the abnormal physio-pathological states associated with high circulatory levels of ADH.

The authors are grateful to the Indian Council of Medical Research for financial assistance.

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(Received September 22, 1976.)