

Central cholinergic and adrenergic mechanisms in the release of antidiuretic hormone

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Summary

1. Studies on the urine outflow, blood ADH concentration and electrolyte excretion were carried out in α -chloralose anaesthetized hydrated dogs; the agonists and antagonists of specific cholinceptors and adrenoceptors were injected by the intracerebroventricular technique, to delineate the role of the C.N.S. receptors in the control of ADH secretion.
2. Central injection of acetylcholine elicited a dose-dependent antidiuretic response which was associated with an increase in the blood ADH titre. Central atropinization partially blocked the antidiuretic response. The remaining antidiuretic response was reversed to a diuretic one by further pretreatment with phenoxybenzamine. The diuretic response thus obtained could be blocked by propranolol.
3. The α -adrenoceptor agonists, phenylephrine and noradrenaline, induced dose-dependent antidiuretic responses with a concomitant rise in blood ADH concentration. Their effect could be blocked by pretreatment centrally with phenoxybenzamine. Low doses of adrenaline induced a diuretic response and a decrease in blood ADH concentration, higher doses elicited a dose-dependent antidiuretic response and increase in the titre of ADH in blood. Central phenoxybenzamine pretreatment reversed the antidiuretic effect of high doses of adrenaline to a diuretic effect which could be blocked by propranolol.
4. Isoprenaline elicited a dose-dependent diuretic response and a decrease in blood ADH titre and propranolol competitively blocked the effect of isoprenaline.
5. It is concluded that central muscarinic cholinceptors and the α -adrenoceptors are concerned in the release of ADH, whereas the β -adrenoceptors are concerned with inhibition of ADH release.

Methods

Sixty-two mongrel dogs of either sex, weighing between 10 to 15 kg, were employed in the study. Animals were fasted for 24 hours but were allowed water *ad lib*. The dogs were anaesthetized with a warm solution of 1% α -chloralose in 0.9% NaCl solution (10 ml/kg) administered intravenously. The diuresis was produced by intravenous infusion of an iso-osmotic solution containing 1.8% dextrose and 0.14% sodium chloride and to maintain anaesthesia 0.05% α -chloralose was added to the solution. The solution was initially infused at the rate of 1.5 to 4.0 ml/min till a total of 50 ml/kg had been delivered. Thereafter, the rate of infusion was adjusted to exceed slightly the rate of urine flow in order to maintain

the water diuresis (Mills & Wang, 1964). However, in experiments with isoprenaline which induced a diuretic response, hydration was not required and hence, to compensate for the loss of fluid in the urine and to maintain the anaesthesia, a continuous drip of 0.1% α -chloralose in 0.9% saline was delivered at the rate of 1 ml/min.

For collection of urine, both the ureters were cannulated and connected to a Palmer photoelectric drop-recording assembly. One of the femoral arteries was cannulated and blood pressure was recorded by means of a mercury manometer on smoked kymograph paper. One of the external jugular veins was exposed and a polythene cannula was inserted to collect blood for estimation of the ADH content.

Drugs were administered intracerebroventricularly (i.c.v.) through a cannula implanted by the technique described by Bhargava & Tangri (1959). The correct placement of the cannula was confirmed at autopsy. The volume of fluid injected (drug solution followed by 0.25 ml saline) never exceeded 0.5 ml; in control experiments an equal volume of saline did not influence the urine flow.

Blood taken from the jugular vein was extracted for ADH estimation on a column of XE-64 resin according to the method of Weinstein, Berne & Sachs (1960) as modified by Yoshida, Motohashi, Ibayashi & Okinaka (1963). By this method the recovery of ADH obtained averaged 80%. The ADH in the eluate was assayed in rats by the technique of Jeffers, Livezy & Austin (1942) as modified by Dicker (1953). Arginine-vasopressin (Pitressin, Parke-Davis) was used as a standard. Necessary corrections in the ADH level for the loss of hormone during extraction procedure were made.

Electrolytes (Na and K) were estimated in urine by flame photometry (Wootton, 1964) and their rate of excretion (mEq/min) was calculated.

Significance of the observations in this study was ascertained by applying Student's *t* test.

Drugs

Acetylcholine chloride (E. Merck), (–)-phenylephrine hydrochloride (Sigma), (–)-noradrenaline hydrochloride (Sigma), (–)-adrenaline hydrochloride (Sigma), dopamine hydrochloride (Sigma), (±)-isoprenaline sulphate (B.W.), phenoxybenzamine (S.K.F.), atropine sulphate (E. Merck), (±)-propranolol (I.C.I.) and vasopressin (Pitressin, Parke-Davis) were the drugs employed in the study.

Results

Effect of acetylcholine (ACh)

Intracerebroventricular injection of ACh (10–500 μ g) produced a decrease in the rate of urine flow which was associated with a rise in blood ADH concentration (Fig. 1). The antidiuretic response was found to be dose-dependent and a linear dose-response relationship was obtained; there was a corresponding increase in the blood ADH levels. The antidiuretic effect of ACh began within 10 min of the injection; the peak effect was observed within 20 minutes. The rate of urine flow returned to the control level in about 60 minutes. The duration of the ACh-induced antidiuretic response was also dose-dependent. Higher doses of

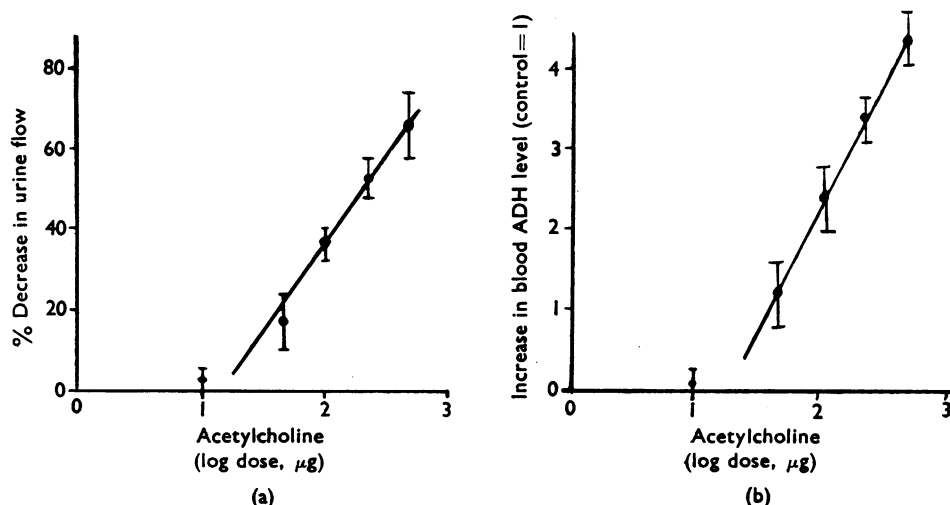


FIG. 1. Effect of graded doses of acetylcholine (i.c.v.) on the urine outflow (a) and blood ADH concentration (b) in dog. Acetylcholine induced an antidiuretic response accompanied by a rise in blood ADH titre. Note the linearity of both the log dose-response regression lines.

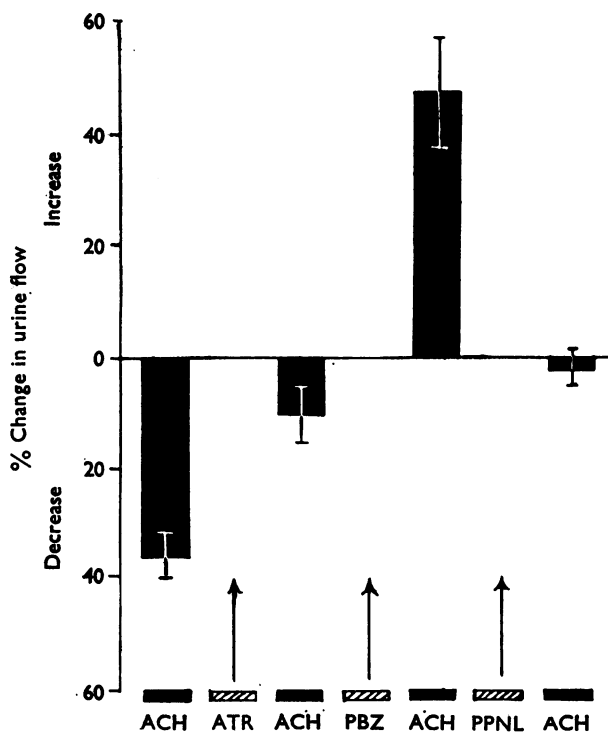


FIG. 2. Histogram depicting changes in the acetylcholine (ACh)-induced antidiuretic response following the sequential administration of receptor blocking agents. ACh ($100 \mu\text{g}$ i.c.v.) in the untreated dogs elicited an antidiuretic response; central pretreatment with atropine (ATR, 2 mg) significantly reduced this response. Further treatment with phenoxybenzamine (PBZ, 2 mg i.c.v.) not only completely blocked the antidiuretic response to ACh but also converted it to a diuretic response. Subsequent treatment with propranolol (PPNL, 2 mg i.c.v.) completely blocked this diuretic effect of ACh.

ACh (100–500 μg) elicited a short lasting (10 min) pressor response (10–15 mmHg) in addition to the antidiuretic effect.

In centrally atropinized (2.0 mg) animals, the antidiuretic response to ACh was significantly reduced ($P < 0.05$). Furthermore, in dogs that had combined pretreatment with atropine and phenoxybenzamine (2.0 mg i.c.v. each), central administration of ACh consistently produced a diuretic effect which could be blocked by propranolol (2.0 mg i.c.v.). The sequence of effects of such a study is depicted in Fig. 2.

Effect of phenylephrine and noradrenaline

Phenylephrine and noradrenaline (10–200 μg i.c.v.) produced a dose-dependent antidiuresis accompanied by a rise in ADH titre in the blood from the jugular vein. The plot of log-dose and per cent reduction in the urine flow or increase in the ADH level gave linear regression lines (see Fig. 3). The dose-response regression lines for phenylephrine and noradrenaline were parallel. The time-course of the antidiuretic effects with phenylephrine and noradrenaline was similar to that observed with ACh. The antidiuretic effects of phenylephrine and noradrenaline were blocked by prior treatment with phenoxybenzamine (2.0 mg i.c.v.). Phenylephrine as well as noradrenaline (100–200 μg i.c.v.) evoked a depressor response (10–15 mmHg) which lasted for 5 to 10 minutes.

Effect of adrenaline

Adrenaline, in low doses (1–5 μg i.c.v.), consistently elicited a diuretic response. However, with 10 μg adrenaline a variable effect on the urine flow was observed; out of 13 experiments, diuresis was observed in 8 and antidiuresis in 5 animals. The diuretic effect obtained with the low doses of adrenaline was always accompanied by a fall in the blood ADH level.

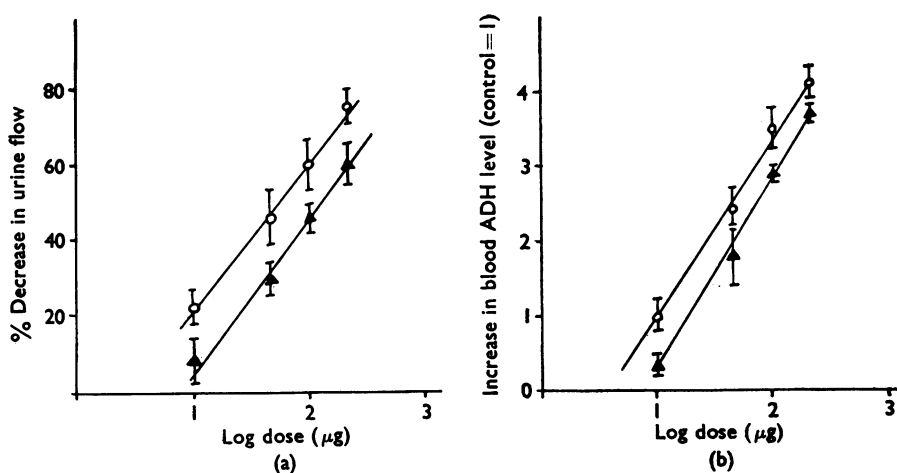


FIG. 3. Effect of intracerebroventricular administration of graded doses of phenylephrine and noradrenaline on the urine outflow (a) and blood ADH concentration (b) in dog. The α -adrenoceptor agonists produced a dose-dependent decrease in urine outflow and a concomitant increase in blood ADH titre. Note the parallelism of log dose-response regression lines.

Higher doses of adrenaline (50–500 μg i.c.v.), on the contrary, always evoked an antidiuretic response which was accompanied by a rise of the ADH titre in the jugular vein blood. A linear dose-response relationship was observed with this dose range of adrenaline. The time-course of the antidiuretic response and the blood pressure changes with higher doses of adrenaline were similar to those with phenylephrine and noradrenaline (i.c.v.). The changes in blood ADH levels obtained with the different doses of adrenaline are represented in Fig. 4. It is significant that low doses of adrenaline (1–5 μg) decreased the blood ADH titre, the dose of 10 μg adrenaline showed marked variation in the titre whereas the higher doses (100–500 μg) consistently raised the blood ADH level. Pretreatment of animals with phenoxybenzamine had no effect on the response to low doses of adrenaline but the effect of high doses of adrenaline was blocked (see Fig. 4). However, the effect of the low doses of adrenaline could be blocked by propranolol (2.0 mg i.c.v.). Combined pretreatment with propranolol and phenoxybenzamine (2.0 mg each, i.c.v.) blocked the effects of all doses of adrenaline tested (1.0–500 μg). These changes in the blood ADH level were reflected in changes in the urine output.

Effect of isoprenaline

Isoprenaline (1.0–20 μg) on intracerebroventricular administration consistently produced a diuretic response. There was a linear dose-response relationship.

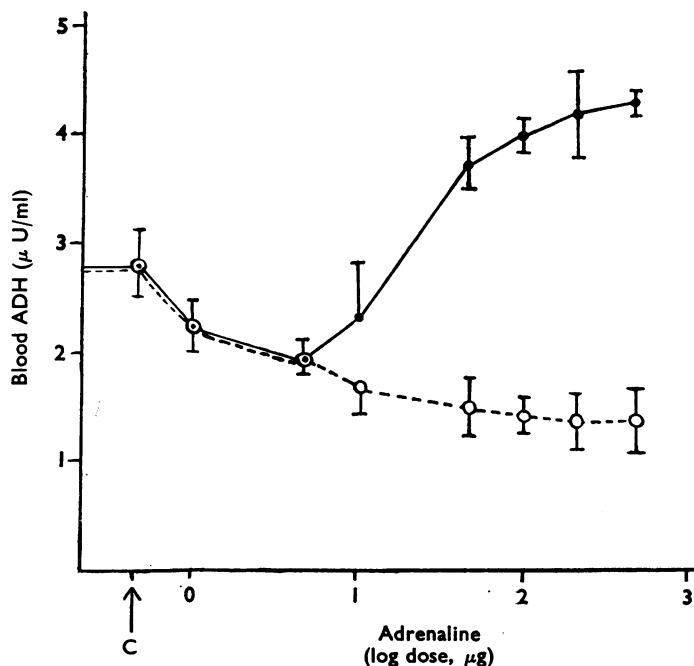


FIG. 4. Effect of graded doses of adrenaline (i.c.v.) on the blood ADH concentration in dogs. Adrenaline (1–5 μg) produced a decrease in the blood ADH; with adrenaline 10 μg although the mean ADH concentration was less than the control (C), in individual experiments the response was either diuresis or antidiuresis (note the large standard error of the mean). Higher doses of adrenaline (50–500 μg) in normal dogs consistently produced an increase in the blood ADH. In phenoxybenzamine (2 mg i.c.v.)-treated dogs, higher doses of adrenaline induced a decrease in the blood ADH titre.

With isoprenaline 20 μg , the increase in the urine flow was observed within 10 min, reached a peak within 20 min and gradual recovery was obtained within 30–60 minutes. With lower doses of isoprenaline, the time course of the diuretic response was shortened. Associated with the increase in urine flow induced by i.c.v. isoprenaline there was a dose-dependent decrease in the ADH titre of the jugular vein blood. Propranolol (2 mg i.c.v.) given 30 min prior to isoprenaline produced a parallel shift of the diuretic dose-response curve (i.c.v.) to the right, indicating a selective action of the isoprenaline on central β -adrenoceptors (see Fig. 5).

Effect of dopamine

Intracerebroventricular administration of dopamine (100 μg –1.0 mg) produced no significant effect on the urine flow. The ADH titre in the jugular vein blood also showed no significant change ($P>0.05$) after dopamine.

Effect of 5-hydroxytryptamine

5-Hydroxytryptamine (100–500 μg i.c.v.) did not affect the urine flow or blood ADH concentration of dogs ($P>0.05$).

Effect on urinary electrolytes

Acetylcholine, 5-hydroxytryptamine and all the adrenergic drugs tested did not produce any change in the excretion rate of sodium and potassium in the urine ($P>0.05$).

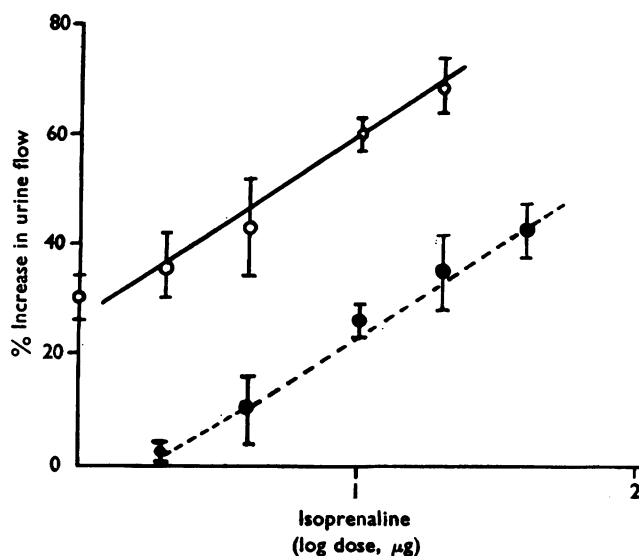


FIG. 5. Effect of graded doses of isoprenaline (i.c.v.) on the urine outflow in normal and propranolol-treated dogs. Isoprenaline elicited a dose-dependent increase in the urine outflow. There was a parallel shift to the right of the dose-response regression line of isoprenaline in the propranolol (2 mg i.c.v.)-treated dogs.

TABLE 1. Effect of receptor blocking agents on blood ADH concentration, urine outflow and blood pressure in dog

Drug	Change in ADH (control = 3.4 ± 0.2 µU/ml)	Urine outflow			Blood pressure	
		% Change	Onset (min)	Peak (min)	Change (mmHg)	Duration (min)
Atropine (2 mg)	(-) 1.8 ± 0.2	(+) 26.1 ± 2.5	1-4	15-20	(+) 10-15	5-10
Phenoxybenzamine (2 mg)	(-) 2.2 ± 0.6	(+) 32.6 ± 3.8	2-5	20-35	(±) No change	—
Propranolol (2 mg)	(±) No change	(+) 15.8 ± 1.6	1-4	5-10	(+) 40-60	15-20
Early	(+) 2.8 ± 0.4	(-) 56.1 ± 5.2	20-25	30-40	(-) 5-10	20-40
Late						

(+) = Increase; (-) = decrease

Effect of blocking agents

The classical blocking agents were employed to block the respective receptors during the course of the study. It was, however, necessary to study the effects of these agents *per se* on the urine output, blood ADH level and urinary electrolyte excretion. The results of the study are summarized in Table 1. The effect of i.c.v. atropine, a muscarinic receptor blocking agent, and phenoxybenzamine, an α -adrenoceptor blocking agent, was an increase in the urine output, decrease in ADH concentration and no change in the electrolyte excretion. Propranolol, which blocks β -adrenoceptors, on the other hand, produced an early transient diuretic response followed by a prolonged antidiuretic response. The early diuretic response to i.c.v. propranolol may be the result of a marked pressor response, since there was no change in the ADH concentration of blood and there was an increase in rate of urinary electrolyte excretion. Associated with the late antidiuretic response to propranolol, there was an increase in the blood ADH concentration and no change in the urinary electrolyte excretion.

Discussion

Evidence is presented to show that the afferents to the supraoptic nucleus are cholinergic as well as monoaminergic. The distribution of both choline-acetylase (Feldberg & Vogt, 1948) and acetylcholinesterase (Abrahams, Koelle & Smart, 1957; Koelle & Geesey, 1961) in the hypothalamoneurohypophysial system indicates that cholinergic fibres terminate at the supraoptic and paraventricular nuclei. Pickford (1939, 1947) and Pickford & Watt (1951) showed that ACh induced inhibition of urine flow by liberation of ADH in unanaesthetized dogs in water diuresis. However, the nature of the cholinceptor at the supraoptic nucleus was not determined since atropinization did not completely block the antidiuretic effects of ACh. Furthermore, intravenous hexamethonium did not block the antidiuretic effect of nicotine in doses which were sufficient to block the pressor and convulsant actions of the drug (Bisset & Walker, 1957). It is indeed possible that the drug failed to penetrate the blood brain barrier.

In the present study, intracerebroventricular administration of ACh elicited a dose-dependent antidiuretic response which was associated with a rise of ADH titre in the jugular vein blood. Central atropinization significantly reduced the antidiuretic effect of ACh. The residual antidiuretic effect of ACh observed in the atropinized animals, could be blocked by central administration of phenoxybenzamine. In dogs given combined pretreatment with atropine and phenoxybenzamine, i.c.v., ACh consistently elicited a diuretic response which could be blocked by propranolol. These results would seem to indicate a dual mechanism of action of ACh. Thus the antidiuretic effect of ACh may be partially operating through the central cholinergic (muscarinic) receptors and partially through the release of catecholamines. It has been shown that ACh releases catecholamines in the periphery (Douglas & Rubin, 1961) and centrally (Phillips, Heyd & Burger, 1970). We suggest, therefore, that besides the cholinergic synapse at the supraoptic nucleus there are adrenergic synapses which also modulate the release of ADH. Since the α -adrenoceptor blocker, phenoxybenzamine, reverses the antidiuretic effect of i.c.v. ACh in atropinized animals and the subsequent diuretic response is blocked by the specific β -adrenoceptor blocker, propranolol, it appears

that the α -receptors are concerned with the release and β -receptors with the inhibition of the release of ADH. These conclusions have been strengthened by our subsequent studies with the adrenergic agents.

More recently sufficient evidence has accumulated in favour of monoaminergic transmission in the central nervous system. The hypothalamus is very rich in monoamines, namely noradrenaline, adrenaline, dopamine and 5-hydroxytryptamine (Twarog & Page, 1953; Vogt, 1954; Carlsson, 1959). The distribution of the monoamines, and their synthesizing and inactivating enzymes have been mapped out in the central nervous system (Bertler, 1960). Histochemical localization of the amines has been accomplished (Fuxe, Hökfelt & Ungerstedt, 1969) which suggests a neurotransmitter role of these monoamines. The supraoptic and paraventricular nuclei are richly innervated with adrenergic nerve terminals (Carlsson, Falck & Hillarp, 1962; Fuxe, 1965). Adrenoceptive drugs, on intravenous administration have been shown to facilitate (Dearborn & Lasagna, 1952; Houck, 1951; Eränkö & Karvonen, 1952) as well as inhibit the release of ADH induced by nociceptive stimuli (O'Connor & Verney, 1945) and acetylcholine (Duke & Pickford, 1951). The α -adrenoceptor blocking agents have been shown to block the antidiuretic response elicited by electrical stimulation of the hypothalamus or the central cut end of the ulnar nerve (Fang *et al.*, 1962; Mills & Wang, 1964). All these observations are in support of a central adrenergic mechanism in the control of ADH release.

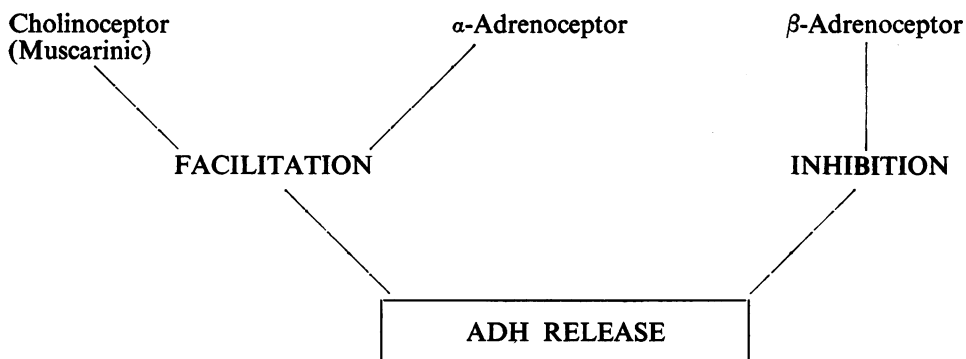
In the present study, we observed that intracerebroventricular injections of phenylephrine (10–200 μ g), noradrenaline (10–200 μ g) and high doses of adrenaline (50–500 μ g) elicited a dose-dependent decrease in the urine output and a concomitant increase in the ADH concentration of blood. The antidiuretic effect of these α -agonists could be completely blocked by central pretreatment with phenoxybenzamine. Thus, it may be concluded that the central α -adrenoceptors are concerned in the release of ADH.

On the other hand, provided that the animals had been given a preliminary dose of phenoxybenzamine, the high doses of adrenaline (50–500 μ g) induced a diuretic response. An inhibition of the release of ADH by low doses of intravenous adrenaline (10–15 μ g) was reported earlier (O'Connor & Verney, 1945; Duke & Pickford, 1951). Similarly, in the present study, i.c.v. adrenaline (1–10 μ g) also elicited the diuretic response. Furthermore, the diuretic effect of adrenaline could be blocked by central administration of propranolol (2 mg i.c.v.). This suggests the participation of β -adrenergic receptors in the diuretic response. Further proof was obtained from the studies with the specific β -receptor agonist, isoprenaline. Since isoprenaline (1–20 μ g i.c.v.) elicited a dose-dependent increase in urine flow and decrease in the plasma concentration of ADH and there was a parallel shift of dose-response regression lines to the right in the presence of propranolol, it may be concluded that the central β -adrenoceptors are concerned in the inhibition of ADH release.

Since intracerebroventricular administration of dopamine (up to 1 mg) and 5-hydroxytryptamine (100–500 μ g) had no significant effect on the urine outflow or ADH level, it is concluded that dopaminergic and tryptaminergic afferents to the supraoptic nucleus are not concerned in the release of ADH. The tryptaminergic mechanism in the regulation of ADH secretion was also ruled out by Dahlström & Fuxe (1965). Similarly, the dopaminergic mechanism, although concerned in the

release of gonadotrophins of the anterior pituitary does not seem to play any part in the control of ADH secretion (Fuxe & Hökfelt, 1966).

The precise organization of the cholinergic and adrenergic fibres in relation to the supraoptic nucleus is not clear. Feldberg (1950) on the basis of the work of Pickford (1939, 1947) suggested that the afferent fibres to the supraoptic nucleus are cholinergic and the last neurones by which the nerve impulses are conveyed to the neurohypophysis are non-cholinergic. Dearborn & Lasagna (1952) suggested that ACh and adrenaline may compete for the same receptor sites to stimulate ADH secretion. However, current histochemical and pharmacological data do not favour these views. From the present study it is possible to represent schematically the cholinergic and adrenergic mechanisms operating at the supraoptic nucleus which may be concerned in the release of ADH, thus:



Finally, it may be concluded that the central cholinoceptors involved in the release of ADH are muscarinic in nature. The central adrenergic system seems to have a dual function in the control of ADH release. The α -adrenoceptors are responsible for the release of ADH whereas the β -adrenoceptors inhibit the ADH release.

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