Cardiovascular effects of central injection of acetylcholine in anaesthetized dogs: a role for vasopressin release

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- 1 The effect of an intracisternal injection of $20 \mu\text{g kg}^{-1}$ of acetylcholine was studied on systolic and diastolic blood pressures, heart rate, and plasma levels of noradrenaline, adrenaline, vasopressin, plasma renin activity and atrial natriuretic factor in chloralose-anaesthetized dogs, 8 of which were normal and 7 with diabetes insipidus (deprived of vasopressin secretion by surgical lesion of the hypothalamoneurohypophyseal system).
- 2 Acetylcholine significantly increased systolic and diastolic blood pressures in both groups of animals. However, the rise in blood pressure was significantly shorter lived in the dogs with diabetes insipidus.
- 3 Acetylcholine significantly increased plasma levels of noradrenaline but not adrenaline in control animals and in dogs with diabetes insipidus. Noradrenaline and adrenaline responses after acetylcholine were not different in the two groups of animals.
- 4 Acetylcholine induced a significant increase in vasopressin plasma levels only in control animals while in dogs with diabetes insipidus vasopressin remained at nearly undetectable levels.
- 5 Acetylcholine significantly increased atrial natriuretic factor plasma levels only in control dogs.
- 6 Although plasma renin activity increased in both groups of animals after the i.c. injection of acetylcholine, this change was not significant in any group.
- 7 These results suggest that, in the anaesthetized dog, the central injection of acetylcholine induces a rise in blood pressure through both an increase in sympathetic outflow and a release of vasopressin.

Introduction

Since the early observation by Shu *et al.* (1935) of the rise in blood pressure elicited by an intracisternal injection of acetylcholine in the anaesthetized dog, many studies have described the cholinergically mediated pressor response induced by the central administration of acetylcholine or related drugs (cholinoceptor agonists or acetylcholinesterase inhibitors) in several species including rat, cat and dog (for review see Brezenoff & Giuliano, 1982). In contrast, the peripheral mechanisms mediating such cholinergic cardiovascular effects remain poorly known. The main one generally put forward is a centrally-evoked cholinergic activation of the sympathetic outflow; however, beside this enhancement of the peripheral noradrenergic activity, the putative effects of other systems remain unclear: a role for vasopressin release is debated and, to our knowledge, the possible role of other peripheral vasoactive systems like plasma renin or atrial natriuretic factor has not been investigated.

The purpose of the present study was to investigate the respective roles of peripheral noradrenaline, adrenaline, vasopressin, plasma renin activity and atrial natriuretic factor in mediating the central cholinergic pressor response. Therefore, we studied the cardiovascular responses and the plasma level variations of noradrenaline, adrenaline, vasopressin, plasma renin activity and atrial natriuretic factor elicited by an intracisternal administration of acetylcholine ($20 \mu\text{g kg}^{-1}$) in anaesthetized control dogs and in dogs with diabetes insipidus induced by surgical section of the supraoptico-hypophyseal tract.

Methods

General procedure

The experiments were performed between 08 h 00 min and 12 h 00 min in mongrel dogs of either sex weighing 15 to 24 kg.

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The dogs were deprived of food the morning of the experiment but had free access to water *ad libitum* in order to be normally hydrated. Animals were anaesthetized with chloralose (100 mg kg^{-1} , i.v.), intubated and ventilated with an Ideal Palmer respirator. Animal temperature was maintained constant with an electric blanket. A catheter introduced into the left femoral artery was connected to a Statham P23 Db transducer to monitor systolic and diastolic blood pressures. Heart rate was counted on the electrocardiogram (lead II). These parameters were continuously recorded on a Philips recorder.

A single dose of $20 \mu\text{g kg}^{-1}$ of acetylcholine chloride (Lemotte and Boinot Labs) dissolved in a 0.9% w/v NaCl solution (saline) was injected into each dog by the intracisternal (i.c.) route. I.c. injections were performed with a 18 gauge needle introduced percutaneously. This dose was chosen after preliminary experiments because it induces, according to the present protocol, a reproducible rise in blood pressure of about 30 mmHg. This effect was blocked by intracisternal atropine ($1 \mu\text{g kg}^{-1}$) (unpublished data). The volume of the injection was no larger than 0.5 ml; before injection of acetylcholine, each animal received a preliminary i.c. injection of the same volume of saline to confirm the absence of cardiovascular effects of a sham injection.

A second catheter, introduced into the right femoral artery, was used for the collection of blood samples in order to measure noradrenaline, adrenaline, vasopressin, plasma renin activity and atrial natriuretic factor plasma levels. Such blood samples were collected 5 and 1 min before and 2, 10, and 30 min after the i.c. injection of acetylcholine.

Dogs with diabetes insipidus: surgical procedure

Dogs were deprived of vasopressin secretion by surgical lesion of the hypothalamo-neurohypophyseal system. The general procedure for inducing diabetes insipidus in dogs has been described previously (Montastruc *et al.*, 1980). Briefly, under intravenous sodium thiopentone anaesthesia (12.5 mg kg^{-1}), the hypothalamus was reached after cutting away the soft palate and collapsing the bone along the sphenothmoidal suture. Sectioning of the supraoptico-hypophyseal tract was

performed with a cataract lancet between the adenohypophysis and the supraoptic nucleus. The aperture was then filled with acrylic resin. Increases in urine excretion (+2 to 6 l daily in different animals; $P < 0.05$ in comparison with normal dogs) were seen 15 to 20 days after surgery. Surgery was performed several months (at least three) before the present study. The selectivity of the surgical lesion which induces the diabetes insipidus is illustrated by previous work from our laboratory (Baisset & Montastruc, 1957), showing that adrenalectomy improves the polyuric-polydipsic syndrome of these animals. This effect is ascribed to the suppression of the diuretic effects of cortisol and suggests that the surgical procedure does not interfere with the integrity of the adenohypophyseal-adrenocortical axis.

Measurement of plasma hormone levels

Blood samples were obtained from the femoral artery and the volume (10 ml) was immediately replaced by 10 ml isotonic saline. All hormonal measurements were assessed blind. For catecholamine determination, blood was collected in lithium-heparin tubes containing 10 mM sodium metabisulphite; for vasopressin and plasma renin activity determinations, blood was collected in lithium heparin-tubes and for atrial natriuretic factor blood was collected in tubes containing EDTA. Blood samples were then centrifuged at 2000 g for 10 min at 0°C; plasma was stored at -80°C.

Catecholamines were selectively isolated from the sample at 0°C, in the dark, by adsorption to activated alumina, then eluted with 0.1 M perchloric acid. Dihydroxybenzylamine was used as an internal standard to monitor recovery from this extraction step. Noradrenaline and adrenaline were assayed by high pressure liquid chromatography using electrochemical (amperometric) detection: the working electrode potential was set at 0.65 V against a Ag-AgCl reference electrode. Catecholamines were separated on a C_{18} Waters column (3.9×150 mm) at a constant flow rate of 1 ml min⁻¹. The electrochemical detector response was linear for concentrations ranging from 0.17 nM to 0.60 μ M; $r = 0.997$ for noradrenaline, $r = 0.922$ for adrenaline. In these conditions the detection limit was 0.3 nM. Inter-assay coefficients of variation for plasma catecholamines performed over 5 days were, for the high levels, 11% for noradrenaline and 12% for adrenaline. The intra-assay coefficients of variation over three assays were 1% for noradrenaline and 7% for adrenaline.

Other hormones were measured by radioimmunoassay. For vasopressin the radioimmunoassay used after bentonite extraction (Skowsky *et al.*, 1974) had a sensitivity of 0.3 pg ml⁻¹. The recovery of vasopressin was 75% and intra and interassay coefficients of variation were 3 and 11%, respectively. The antibodies were a gift of L. Keil (Keil & Severs, 1977). Atrial natriuretic factor radioimmunoassay was made after SEPAK extraction as previously described (Gutkowska *et al.*, 1986). The antibodies were a gift of J. Gutkowska. The recovery for added atrial natriuretic factor was 85%. Intra and interassay coefficients of variations were 10%, and sensitivity 1.2 pg ml⁻¹. Plasma renin activity was measured as previously described (Vincent *et al.*, 1972) with antibodies raised in the laboratory. Intra and interassay coefficients of variations were 1% and 2.5% respectively and sensitivity 20 ng l⁻¹ min⁻¹.

Statistical analysis

Non-parametric tests were used for statistical comparisons. The Mann-Whitney U test was performed to compare the mean values of cardiovascular parameters and hormonal plasma levels between the group of control animals and the group of dogs with diabetes insipidus. This test was also used to compare the mean cardiovascular and hormonal changes induced by the i.c. injection of acetylcholine in the 2 groups of animals. Friedman's test was performed in each group to compare cardiovascular and hormonal values before and after the i.c. injection of acetylcholine.

Results

Eight control dogs (5 females, 3 males, mean weight: 19.4 ± 2.1 kg) and 7 animals with diabetes insipidus (4 females, 3 males, mean weight: 22.3 ± 1.7 kg) were studied in the present experiments.

Cardiovascular responses to intracisternal injections of acetylcholine

Systolic and diastolic blood pressures and heart rate were similar under basal conditions in control animals and dogs with diabetes insipidus (Table 1). Although mean values of systolic blood pressure appeared to be lower in dogs with dia-

Table 1 Mean systolic and diastolic blood pressure and heart rate of control animals and dogs with diabetes insipidus 5 and 1 min before and 2, 10 and 30 min after the intracisternal injection of acetylcholine

Acetylcholine (20 μ g kg ⁻¹ , i.c.)		Before injection		After injection			Friedman's test
		5 min	1 min	2 min	10 min	30 min	
Control animals (n = 8)	Systolic blood pressure (mmHg)	163 $\pm$ 15	167 $\pm$ 16	191 $\pm$ 16	187 $\pm$ 17	174 $\pm$ 17	$P < 0.001$
	Diastolic blood pressure (mmHg)	68 $\pm$ 4	69 $\pm$ 4	91 $\pm$ 4	91 $\pm$ 4	76 $\pm$ 5	$P < 0.01$
	Heart rate (min ⁻¹)	116 $\pm$ 9	112 $\pm$ 9	119 $\pm$ 10	123 $\pm$ 8	120 $\pm$ 10	NS
Animals with diabetes insipidus (n = 7)	Systolic blood pressure (mmHg)	142 $\pm$ 9	142 $\pm$ 9	169 $\pm$ 12	157 $\pm$ 9	143 $\pm$ 9	$P < 0.01$
	Diastolic blood pressure (mmHg)	71 $\pm$ 5	71 $\pm$ 5	85 $\pm$ 4	79 $\pm$ 4	72 $\pm$ 5	$P < 0.01$
	Heart rate (min ⁻¹)	122 $\pm$ 13	123 $\pm$ 13	130 $\pm$ 12	135 $\pm$ 12	130 $\pm$ 13	NS

There was no difference between control animals and dogs with diabetes insipidus at baseline, before the injection (Mann and Whitney U test). Acetylcholine induced a significant increase in systolic and diastolic blood pressures in both groups of animals (Friedman's test). This response was shorter lived in the dogs with diabetes insipidus: 30 min after acetylcholine, blood pressure had returned to basal values in the dogs with diabetes insipidus but not in the control group (see Figure 1).

betes insipidus, this difference was not significant and this observation agrees with previous results from our laboratory (Montastruc & Dang-Tran, 1984).

In the control group, systolic and diastolic blood pressures increased after the i.c. injection of acetylcholine within 2 min ($P < 0.001$ and $P < 0.01$, respectively). Heart rate slightly and non significantly increased after the i.c. injection of acetylcholine (Table 1).

In the group of dogs with diabetes insipidus, systolic and diastolic blood pressure significantly increased ($P < 0.01$). The moderate increase which was observed in heart rate was not significant (Table 1).

When the cardiovascular responses of the dogs with diabetes insipidus were compared to those of the controls, the initial rise in systolic and diastolic blood pressures (2 min after the i.c. injection of acetylcholine) were similar in both groups (Figure 1). However the pressor effect was shorter in duration in the group of animals with diabetes insipidus: 30 min after the i.c. injection, the mean increase in systolic blood pressure induced by acetylcholine was significantly smaller in the dogs with diabetes insipidus than in the control animals ($P < 0.05$); a difference was also observed in the changes in diastolic blood pressure, the increase being significantly smaller in the animals with diabetes insipidus 10 and 30 min after the i.c. injection of acetylcholine ($P < 0.05$). No difference existed in the changes in heart rate between the two groups of animals.

Effects of i.c. injections of acetylcholine on hormonal plasma levels (Table 2)

Noradrenaline and adrenaline plasma levels were assessed only 5 min before and 2 and 30 min after the i.c. injection of acetylcholine. Vasopressin, plasma renin activity and atrial natriuretic plasma levels were assessed 5 and 1 min before and 2, 10 and 30 min after the i.c. injection of acetylcholine.

Before the injection of acetylcholine, the basal plasma levels of noradrenaline, adrenaline, plasma renin activity and atrial natriuretic factor were not significantly different in control dogs and animals with diabetes insipidus. On the contrary, as expected, vasopressin plasma levels were nearly undetectable in the group of animals with diabetes insipidus and the difference was highly significant from the control group ($P < 0.01$).

Figure 1 Mean increases in systolic and diastolic blood pressures in control animals (hatched columns) and dogs with diabetes insipidus (open columns) 2, 10 and 30 min after the intracisternal injection of $20 \mu\text{g kg}^{-1}$ of acetylcholine (ACh) (basal values 5 and 1 min before the injection of acetylcholine). Notice that, in dogs with diabetes insipidus, the rise in systolic blood pressure observed 30 min after the i.c. injection of acetylcholine was significantly smaller than in control animals; the same difference was also observed for the rise in diastolic blood pressure which was significantly smaller in the dogs with diabetes insipidus 10 and 30 min after the i.c. injection of acetylcholine (* $P < 0.05$, Mann-Whitney U test).

The i.c. injection of acetylcholine significantly increased noradrenaline plasma levels in control animals and in dogs with diabetes insipidus ($P < 0.02$) (Table 1). This increment did not differ from one group to the other.

Table 2 Mean plasma levels of noradrenaline, adrenaline, vasopressin, atrial natriuretic factor and plasma renin activity in control animals and dogs with diabetes insipidus 5 and 1 min before and 2, 10 and 30 min after the intracisternal injection of acetylcholine

Acetylcholine ($20 \mu\text{g kg}^{-1}$, i.c.)		Before injection		After injection			Friedman's test
		5 min	1 min	2 min	10 min	* 30 min	
Control animals	Noradrenaline (pg ml^{-1})	376 $\pm$ 94	—	485 $\pm$ 97	—	436 $\pm$ 133	$P < 0.02$
	Adrenaline (pg ml^{-1})	85 $\pm$ 23	—	92 $\pm$ 29	—	77 $\pm$ 20	NS
	Vasopressin (pg ml^{-1})	13.5 $\pm$ 6.4	13.7 $\pm$ 4.4	41.3 $\pm$ 9.1	53.5 $\pm$ 10.8	35.1 $\pm$ 6.4	$P < 0.01$
	Atrial natriuretic factor (pg ml^{-1})	102 $\pm$ 15	108 $\pm$ 13	134 $\pm$ 13	145 $\pm$ 23	126 $\pm$ 16	$P < 0.02$
	Plasma renin activity ($\text{ng l}^{-1} \text{ min}^{-1}$)	150 $\pm$ 36	167 $\pm$ 45	175 $\pm$ 52	201 $\pm$ 57	200 $\pm$ 46	NS
Animals with diabetes insipidus	Noradrenaline (pg ml^{-1})	191 $\pm$ 30	—	364 $\pm$ 52	—	304 $\pm$ 123	$P < 0.02$
	Adrenaline (pg ml^{-1})	86 $\pm$ 35	—	110 $\pm$ 44	—	161 $\pm$ 63	NS
	Vasopressin (pg ml^{-1})	0.5 $\pm$ 0.4	0.6 $\pm$ 0.3	0.7 $\pm$ 0.3	1.4 $\pm$ 0.8	1.4 $\pm$ 0.8	NS
	Atrial natriuretic factor (pg ml^{-1})	101 $\pm$ 9	97 $\pm$ 10	121 $\pm$ 20	128 $\pm$ 11	110 $\pm$ 11	NS
	Plasma renin activity ($\text{ng l}^{-1} \text{ min}^{-1}$)	176 $\pm$ 35	183 $\pm$ 40	191 $\pm$ 47	276 $\pm$ 77	339 $\pm$ 99	NS

At baseline, before the injection, hormonal plasma levels were not significantly different between the two groups of dogs except for vasopressin which was significantly reduced in the dogs with diabetes insipidus ($P < 0.01$, Mann-Whitney U test). Acetylcholine significantly increased noradrenaline plasma levels in both control dogs and animals with diabetes insipidus. Acetylcholine significantly increased vasopressin and atrial natriuretic factor plasma levels only in control dogs. The increases observed in plasma renin activity after acetylcholine did not reach statistical significance (Friedman's test).

No significant effect of acetylcholine was observed on adrenaline plasma levels in either group of dogs (Table 1).

In the control group, the i.c. injection of acetylcholine induced a marked and significant increase in vasopressin plasma levels ($P < 0.01$). In contrast, no change was observed in the group of animals with diabetes insipidus.

The i.c. injection of acetylcholine also significantly increased atrial natriuretic factor plasma levels in the control animals ($P < 0.02$). This effect was not observed in the group of animals with diabetes insipidus.

The i.c. injection of acetylcholine increased the plasma renin activity in both groups of animals but this rise was not significant in any group.

Discussion

The i.c. injection of acetylcholine ($20 \mu\text{g kg}^{-1}$) in anaesthetized dogs induced a significant rise in blood pressure. This is in good agreement with the large number of experiments reporting the same results in different species with different cholinergic agents (acetylcholine, cholinesterase inhibitors, cholinergic agonists) administered through the central route (for review, see Brezenoff & Giuliano, 1982). Conversely, in the present experimental model, the i.c. injection of acetylcholine did not significantly modify the heart rate of the dogs. A slight tachycardia was observed in both groups of animals but this response was not constant and not significant. In fact, in the literature, no clear response can be found and variable heart rate changes, tachycardia as well as bradycardia, have been reported after central injection of cholinergic agents in different species (see Brezenoff & Giuliano, 1982). Anaesthetic conditions and interspecies differences may explain some of these conflicting data.

In control dogs and animals with diabetes insipidus, acetylcholine induced a significant rise in noradrenaline plasma levels. This rise illustrated the well known enhanced peripheral noradrenergic activity observed after central cholinergic stimulation which is abolished by the section of the spinal cord rostral to the spinal sympathetic outflow (Krstić & Djurković, 1978), by chemical ablation of the peripheral sympathetic nervous system by 6-hydroxydopamine (Kaul & Grewal, 1972), ganglionic transmission blockade (Della Bella *et al.*, 1964) or peripheral α -adrenoceptor blockade (Krstić & Djurković, 1978). These experiments demonstrated that an increased sympathetic outflow mediates the cardiovascular effects of central cholinergic activation. In the present experiments, a similar initial rise (2 min after the i.c. injection of acetylcholine) in blood pressure and noradrenaline plasma levels was observed in the control animals and the dogs with diabetes insipidus. This observation suggests that the mechanisms underlying these responses may be similar in the two groups of dogs. However, it must be emphasized that, in these animals, the respective contribution of the vascular conductance and of the cardiac output was not assessed. Therefore the possibility remains that the 'same' change in blood pressure in the two groups of animals was achieved with different contributions from changes in these two factors.

Unlike noradrenaline, adrenaline plasma levels were not significantly modified by the i.c. injection of acetylcholine. In fact, this lack of effect in adrenaline output by the adrenal medulla agrees with the observation that adrenalectomy, adrenal demedullation or denervation of the gland do not affect the rise in blood pressure produced by i.v. physostigmine (see Brezenoff & Giuliano, 1982).

Conflicting reports regarding the ability of phentolamine to block the pressor effect of centrally administered cholinergic drugs raises the question whether factors other than the increase in sympathetic tone could mediate this effect. The role of vasopressin has been suggested by some authors and questioned by others. In our experiments, at rest, vasopressin plasma levels were rather high in control animals. This can be explained by the fact that nearly all anaesthetics release

vasopressin (Heller, 1951). In dogs with diabetes insipidus, the very low plasma levels of vasopressin confirmed the efficacy of the surgical procedure. The present data provided two results which favour a role for vasopressin in mediating part of the central pressor effect of acetylcholine.

First, we observed in control animals that acetylcholine induced significant and prolonged rises in peripheral vasopressin plasma levels. This is consistent with the well documented central cholinergic regulation of vasopressin release *in vitro* (Sladek & Joynt, 1979) and *in vivo* (Kuhn, 1974). It has long been accepted that the level of circulating vasopressin needed to produce a significant pressor response is higher than that required for maximal antidiuretic effect (Sawyer, 1961). In the present experiments, the amount of vasopressin released by the i.c. injection of acetylcholine was compatible with a cardiovascular and pressor effect (Rossi & Schrier, 1986).

A second argument implicating vasopressin in the central pressor cholinergic effect was that the hypertension observed after the i.c. injection of acetylcholine was shorter in dogs with diabetes insipidus than in controls. The role of the baroreflex in explaining this difference must be considered. Brain acetylcholine modulates baroreceptor cardiac reflexes (see Brezenoff & Giuliano, 1982) and reciprocal influences between endogenous vasopressin and baroreflex mechanisms have been pointed out (see Gardiner & Bennett, 1986). It is therefore possible that, in dogs with diabetes insipidus, the shorter duration of the cholinergic pressor response was due to an abnormality of the baroreflex. To our knowledge, the baroreflex has not been studied in dogs with surgical diabetes insipidus. However, previous works from our laboratory (Baisset & Montastruc, 1978) showed that the acute hypertension induced by the depressor buffer nerves section was similar in normal dogs and dogs with surgical hypothalamic diabetes insipidus. Moreover, it was noticed, in the present experiment, that no difference in heart rate changes occurred between the two groups of dogs after the i.c. injection of acetylcholine. These observations indirectly suggest that dogs with surgical diabetes insipidus do not present major alterations in their baroreflex. Conversely, as expected, basal vasopressin plasma levels of dogs with diabetes insipidus were nearly undetectable in these animals and the i.c. injection of acetylcholine did not induce any significant rise of vasopressin. At the same time, the duration of the pressor cholinergic response was significantly shorter in the same animals. These data suggest that, besides a similar increase in the sympathetic outflow in the two groups of animals, the difference in the cholinergic release of vasopressin could explain the longer duration of the pressor response in the normal dogs. This putative role of vasopressin has been variously assessed in the literature: Buccafusco & Brezenoff (1979) reported that in anaesthetized rats, the pressor response to the central injection of carbachol was unaltered by hypophysectomy. Kawashima *et al.* (1987) showed that a vasopressin antagonist did not attenuate the pressor or cardiac effects of physostigmine in normal or hypertensive rats. Such results were not in favour of a significant role of vasopressin. However, several other authors reported data that support the role of vasopressin in mediating some aspects of the central pressor cholinergic response. Varagić & Milić (1957) were the first to propose that a release of vasopressin in pressor amounts might be involved in the cardiovascular response to physostigmine in the rat. Hoffman *et al.* (1977) and Hoffman (1979) also observed pressor responses with antidiuresis after the injection of carbachol into the third ventricle of conscious rats. Bisset & Chowdrey (1984) showed that in the anaesthetized rat, intraventricular injections of carbachol produced a profound antidiuretic response associated with a pressor effect. Although these authors did not provide quantitative data on this question, they concluded that 'since the pressor response to carbachol was absent or attenuated in the Brattleboro rat, this too may be mediated, at least partly, by release of vasopressin'. Thus, it is possible to suggest that the cholinergic-induced

pressor and antidiuretic responses are not independent mechanisms.

It is not clear if the central effects of acetylcholine on vasopressin release and blood pressure share common pharmacological mechanisms or not. It is generally accepted that acetylcholine stimulates vasopressin release from the hypothalamohypophyseal system through nicotinic receptors (Milton & Paterson, 1974) rather than muscarinic ones. In contrast, the nature of the central cholinergic pressor response is said to be mainly of the muscarinic type (see Brezenoff & Giuliano, 1982) and, in our model i.c. atropine abolishes the pressor effect of the central injection of acetylcholine (data not shown). According to these data, the cholinergic cardiovascular and endocrinological responses could then be separable and would correspond to different pharmacological mechanisms. However, several authors have reported that, *in vivo*, atropine was able to block the release of vasopressin (Kuhn, 1974; Bisset & Chowdrey, 1984) and to block simultaneously both hormonal and pressor responses elicited by central cholinergic agents (Hoffmann *et al.*, 1977). Therefore, it is possible that both responses are mediated, at least partly, by muscarinic receptors.

The i.c. injection of acetylcholine induces a significant rise in plasma atrial natriuretic factor in control animals. This rise was smaller and did not reach significance in dogs with diabetes insipidus. This increase in atrial natriuretic factor plasma levels can be regarded as a release in response to the pressor effects of the i.c. injection of acetylcholine. The mechanisms regulating atrial natriuretic factor release from the heart are not well understood. However, several factors are known to induce it, such as an increase in atrial pressure (Lang *et al.*, 1985), noradrenaline and other pressor agents (Garcia *et al.*, 1986; Baranowska *et al.*, 1987). A possible interaction between the endocrine systems of the heart and the pituitary gland has been debated (see Hoffman & Keiser, 1989). A direct role of the adeno-hypophysis has not been clearly demonstrated and there is evidence that our surgical procedure to induce diabetes insipidus spares the adeno-hypophysis (see methods of Baisset & Montastruc, 1957). On the contrary, vasopressin has been shown to stimulate atrial natriuretic factor release directly or secondarily to its effect on blood volume (Manning *et al.*, 1985; Ogawa *et al.*, 1986). Therefore, in our control animals, at least three different factors could explain the rise in atrial natriuretic factor plasma levels: the rises in blood pressure, noradrenaline and vasopressin plasma levels. Furthermore, the importance of vasopressin in the *in vivo* release of atrial natriuretic factor was underlined by the fact that in dogs

with diabetes insipidus, deprived of vasopressin secretion, although acetylcholine moderately increased plasma atrial natriuretic factor, this rise was not significant. This could be due to the lack in such animals of a direct effect of vasopressin or to the shorter pressor response observed in dogs with diabetes insipidus.

In the present experiments, plasma renin activity was not significantly modified by the i.c. injection of acetylcholine in control animals and dogs with diabetes insipidus. This result is somewhat surprising since many morphological, physiological and pharmacological data suggest an involvement of the sympathetic nervous system in the mechanism of renin release (Vander, 1965; Wagermark *et al.*, 1968; for review see Reid *et al.*, 1978) and since the i.c. injection of acetylcholine increased sympathetic outflow of our animals. In urethane-anaesthetized rats, Alexandre *et al.* (1970) reported that physostigmine ($75 \mu\text{g kg}^{-1}$, i.v.) increased both blood pressure and plasma renin activity. This increase in plasma renin activity was attributed to the increased sympathetic activity elicited by the anticholinesterase. We did not observe the same results in dogs. However, although the phenomenon did not reach statistical significance, it can be noticed that plasma renin activity was frequently increased in dogs after the central injection of acetylcholine. Moreover, this response was more pronounced in dogs with diabetes insipidus than in control dogs. This last result can be related to the fact that several investigators have observed that vasopressin inhibits renin secretion (Bunag *et al.*, 1967; Vander, 1968). The increase in renin secretion after intravenous isoprenaline is greater in hypophysectomized dogs than in control animals (Reid *et al.*, 1972), a result ascribed to the fact that isoprenaline is a potent stimulus for vasopressin secretion (Schrier *et al.*, 1972). Therefore, it can be suggested that in our control animals, the release of vasopressin induced by acetylcholine may have acted as an inhibitory factor to renin secretion, a fact which did not occur in dogs with diabetes insipidus.

In conclusion, the present data suggest that, besides an increase in the sympathetic outflow, the rise in blood pressure observed after central stimulation of the cholinergic systems can be related to an increase in vasopressin release, while atrial natriuretic factor and plasma renin activity seem to have no or minor roles.

We wish to acknowledge Mr J.M. Duplantier and Mrs G. Portolan for technical assistance and Mrs P. Bontemps for typing the manuscript.

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(Received November 23, 1989)

Revised March 6, 1990

Accepted March 13, 1990)