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Central cardiovascular effects of acetylcholine in the conscious dog

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1 The effects of central cholinomimetic drugs on cardiovascular and vasoactive hormonal responses (blood pressure, heart rate, catecholamines, vasopressin, atrial natriuretic factor, neuropeptide Y plasma levels and plasma renin activity) were investigated in *conscious* Beagle dogs. For this purpose a catheter was chronically implanted into each dog's cisterna magna to allow repeated central injections in the awake animals.

2 Intracisternal acetylcholine (20 $\mu g\ kg^{-1}$) significantly increased systolic and diastolic blood pressure. These changes were accompanied by an initial short term tachycardia followed by a long lasting bradycardia. Intracisternal acetylcholine also increased noradrenaline, adrenaline and vasopressin plasma levels, decreased plasma renin activity but did not modify plasma levels of neuropeptide Y and atrial natriuretic factor.

3 The effects of acetylcholine were completely abolished by pretreatment with intracisternal injection of the muscarinic antagonist, atropine (5 $\mu g\ kg^{-1}$) but not by the intracisternal injection of the nicotinic antagonist, mecamylamine (25 $\mu g\ kg^{-1}$).

4 The present results demonstrate that there are qualitative and quantitative differences between the central cardiovascular effects of acetylcholine in conscious dogs compared to what we previously reported, using a comparable protocol, in anaesthetized dogs. Under both conditions, we observed a central cholinergically mediated increase in blood pressure secondary to an increase in sympathetic tone and vasopressin release but these responses were shorter (less than 10 min) in the conscious dogs than in anaesthetized dogs (more than 10 min). Moreover, we detected in the response to the central cholinergic stimulation in the conscious dogs a significant increase in plasma adrenaline levels and biphasic changes in heart rate which were not described previously in the anaesthetized dog.

Keywords: Acetylcholine; blood pressure; central cholinergic systems; muscarinic receptor; vasopressin; catecholamine

Introduction

It is well known that central acetylcholine (ACh) increases blood pressure. There are however few recent experimental reports concerning the control of blood pressure and heart rate by central cholinergic systems (for review see Brezenoff & Giuliano, 1982). The role of central cholinergic systems is of practical importance because tacrine, an anticholinesterase with central cholinomimetic properties, has recently been marketed in USA and several European countries for the treatment of a very frequent dementia of the elderly, Alzheimer's disease (Schneider, 1994). Many other cholinomimetics are currently under development for this indication. Cardiovascular effects are therefore likely to occur in patients treated with these drugs and there is a need for a better comprehension of the central cholinergic control of blood pressure regulation.

We have previously reported that in chloralose anaesthetized dogs the intracisternal (i.c.) injection of ACh elicits a pressor response mediated by an increase in sympathetic outflow and vasopressin (AVP) release (Rascol *et al.*, 1990). Much of the published work concerning the cardiovascular effects of the central injection of drugs acting at cholinoceptors have been performed in anaesthetized animals. In fact, anaesthesia can interfere with most of the cardiovascular and hormonal responses produced by the i.c. injection of ACh which include changes in sympathetic and vagal tone (Marty & Reves, 1989), AVP release (Heller, 1951) and the activity of brainstem cholinergic neurones (Bradley & Dray, 1973). It is thus possible that anaesthetics modify the nature of the cholinergic cardiovascular and hormonal responses observed in these studies (for review see Brezenoff & Giuliano, 1982).

The aims of the present study were (1) to investigate the cardiovascular and hormonal changes induced by the i.c. injection of ACh in the conscious dog and (2) to characterize the type of the central cholinoceptors (muscarinic or nicotinic) involved in the responses.

Methods

General procedure

Ten conscious Beagle dogs weighing 10 to 15 kg were trained to remain quiet in a Pavlov-type stand. Surgery was previously performed under general anaesthesia (α-chloralose: initial dose of 80 mg kg^{-1} i.v. followed by supplementary doses if needed to maintain a constant level of anaesthesia during surgery) in order to insert a catheter into the cisterna magna for repeated central injections in the conscious animals. For this purpose, the skin and muscles of the posterior part of the neck were dissected. The distal extremity of the catheter (flexopulmocath) was then inserted into the cisterna magna and sutured to the atlanto occipital membrane and adjacent fascia with acrylic thread. The proper position of the catheter within the cisterna magna was demonstrated by the flow of cerebrospinal fluid into the catheter and by the bradycardia and hypotension elicited by a clonidine injection (1.5 $\mu g\ kg^{-1}$) through the same

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catheter. The catheter was passed subcutaneously so that its tip exited at the back of the neck. Animals had at least three days to recover before being used in an experiment. The surgical procedure was not successful in two dogs. One presented a subarachnoid haemorrhage and the other one removed its intracisternal catheter. Thus, we performed the experiments ($n=24$) in eight dogs.

Dogs were fasted on the morning of each experiment but had free access to water in order to be normally hydrated. A catheter was introduced into the left femoral artery at the beginning of each experiment. It was removed at the end of each experiment. It was connected to a Statham P23Db 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. The collection of experimental data only started 15 to 20 min after the insertion of the femoral catheter to allow cardiovascular parameters to return to basal values after this potentially stressful event.

We studied four treatment protocols: (1) acetylcholine (ACh), (2) atropine (Atro) + acetylcholine, (3) mecamylamine (Meca) + acetylcholine and (4) sham protocols. Each treatment protocol was assessed during six separate experiments. We performed an average of three different (2 to 4) treatment protocols in each dog with an interval of two days between each experiment. Each type of treatment protocol was only performed once in the same dog. Some dogs did not receive the four types of treatment protocols because of technical difficulties. We tested the effects of the i.c. injection of a single dose of 20 $\mu g\ kg^{-1}$; acetylcholine chloride (Lemotte and Boinot labs) dissolved into a 0.9% w/v NaCl solution in the ACh protocol. This dose was known to induce a mean rise in blood pressure of 30 mmHg in anaesthetized dogs according to previous comparable experiments performed in our laboratory (Rascol *et al.*, 1990). The effects of i.c. ACh were assessed on blood pressure (BP), heart rate (HR) and hormonal plasma levels: noradrenaline (NA), adrenaline (Ad), vasopressin (AVP), atrial natriuretic factor (ANF), neuropeptide Y (NPY), plasma renin activity (PRA). These parameters were measured at 3 different times: 1 min before (T−1), 2 min after (T+2) and 10 min after (T+10) the i.c. injection of ACh. In the sham protocol, the dogs received a central injection of a similar volume of saline instead of ACh. In the Atro and Meca protocols, either the muscarinic antagonist, atropine or the nicotinic antagonist, mecamylamine was injected by i.c. route 10 min before the i.c. injection of ACh. The dose of Atro necessary to block the central muscarinic receptors (5 $\mu g\ kg^{-1}$) was chosen according to previous data obtained by our group in anaesthetized dogs (Rascol *et al.*, 1990). The dose of Meca was chosen according to preliminary experiments performed in two anaesthetized dogs demonstrating that the pressor response induced by 200 $\mu g\ kg^{-1}$ of ACh injected intravenously (ganglionic nicotinic effect) was completely blocked by 250 $\mu g\ kg^{-1}$ of Meca injected by the same intravenous route. We used the same ratio between ACh and Meca to choose the necessary dose of Meca (25 $\mu g\ kg^{-1}$) injected by the intracisternal route to block the putative nicotinic effects of 20 $\mu g\ kg^{-1}$ of ACh administered i.c. BP, HR and hormonal plasma levels were measured 1 min before and 5 min after the i.c. injection of the antagonists to assess if they themselves induced any effects on the measured parameters. Ten minutes after the i.c. injection of the antagonist, 20 $\mu g\ kg^{-1}$ of ACh were injected i.c. Cardiovascular and hormonal parameters were then measured at T−1, T+2 and T+10 in relation to this i.c. ACh injection. The volume of each i.c. injection was no larger than 0.5 ml. At the beginning of each experiment, a preliminary i.c. injection of the same volume of saline was performed to test for non-specific cardiovascular effects. At the end of each experiment, clonidine (1.5 $\mu g\ kg^{-1}$) was injected through the catheter and the presence of effects on HR and BP indicated that the catheter remained in the cisterna magna.

Measurement of plasma hormone levels

Blood samples were obtained from the femoral artery. Each sample was immediately replaced by an equivalent volume (8 ml) of isotonic saline. All hormonal measurements were assessed 'blindly'. For catecholamine determination, blood was collected in lithium heparin tubes containing 10 mM sodium metabisulphite. For AVP, NPY and PRA determinations, blood was collected in lithium heparin tubes and for ANF blood was collected in tubes containing EDTA. Blood samples were then centrifuged at 4000 *g* for 15 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 on activated alumina, then eluted with 0.1M perchloric acid. Dihydroxybenzylamine was used as an internal standard to monitor recovery from this extraction step. NA and Ad were assayed by high performance 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 C18 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 μM; $r=0.997$ for NA, $r=0.922$ for Ad. 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 NA and 12% for Ad. The intra-assay coefficients of variation over three assays were 1% for NA and 7% for Ad.

Other hormones were measured by radioimmunoassay. Plasma concentrations of NPY were determined by a sensitive and specific radioimmunoassay (Peninsula, England) using a specific antiserum, which was raised in rabbit against porcine NPY. Plasma samples were incubated with [^{125}I]-NPY and a specific rabbit antiserum. Bound antigen was separated by a second antibody method, and its radioactivity was measured in a gamma counter. The antiserum showed no cross-reactivity to structurally related peptides, such as peptide YY or pancreatic polypeptide (less than 0.003% and 0.002%) respectively. The response was linear for concentrations ranging from 25 to 3000 pmol l^{-1}. The detection limit was 25 pmol l^{-1} as previously reported (Poncet *et al.*, 1991). Day to day variability was 4% and within-run 3%. For AVP, the radioimmunoassay used after bentonite extraction (Skowsky *et al.*, 1974) had a sensitivity of 0.3 pg ml^{-1}. The recovery of AVP was 75% and intra and inter assays coefficients of variation were 3 and 11%, respectively. PRA was measured as previously described (Vincent *et al.*, 1972) with antibodies raised in the laboratory. Intra- and inter-assays coefficients of variation were 1 and 2.5% respectively and sensitivity 20 ng l^{-1} min^{-1}. ANF radioimmunoassay was carried out after SEPAK C18 extraction as previously described (Gutkowska *et al.*, 1986). The recovery of added ANF was 85%. Intra and inter assays coefficients of variation were 10 and 12% respectively and sensitivity 1.2 pg ml^{-1} (Gauquelin & Gharib, 1990).

Statistical analysis

The experimental data were obtained from 24 experiments (6 experiments by treatment protocols) performed in 8 dogs. Analysis of variance (ANOVA) was used to compare the means of the different parameters at time T−1 in the 4 protocols in order to assess if there was any significant intergroup difference on baseline before injecting ACh. ANOVA for repeated measures was used to compare the different parameters at time T−1, T+2 and T+10 in the sham protocol in order to assess if there was any significant non specific effect of saline within this protocol. The paired-sample Student's *t* test was used to compare the means of the different parameters before and after i.c. Atro or i.c. Meca in order to assess if the cholinoceptor antagonists induced any effects by themselves. ANOVA or Kruskal-Wallis test according to homogeneity of variances (Bartlett's test) were used to compare the mean's

variations (Δ) of the different parameters from T−1 to T+2 and T+10 in the 4 protocols in order to assess if there was any significant effect of i.c. ACh in the 4 protocols. The Newman-Keuls test or the Mann-Whitney U test were employed as *post hoc* tests for intergroup comparisons.

Values are expressed as mean ± s.e.mean. The level of significance was accepted for $P<0.05$.

Results

Table 1 summarizes cardiovascular and hormonal values at T−1, T+2 and T+10 in the four protocols.

Baseline

Cardiovascular and hormonal parameters were not significantly different at time T−1 in the 4 protocols (Table 1).

Effects of sham injections

Saline induced no behavioural change. Saline induced no significant change in any parameters at T+2 and T+10 except for AVP plasma levels. AVP plasma levels increased by +6±2 pg ml^{-1} at T+10. This moderate but significant increase ($P<0.05$) was probably due to the blood volume removed to collect the previous blood samples at T−1 and T+2.

Effects of the i.c. injection of ACh on cardiovascular responses

ACh (Figure 1) induced behavioural changes in most animals. These changes were characterized by an initial increase in alertness with licking and swallowing. This behaviour lasted less than 1 min and was followed by a prolonged (>10 min) period of quietness and drowsiness.

Blood pressure At T+2, the mean change in systolic blood pressure (Δ syst BP) and diastolic blood pressure (Δ diast BP) were significantly different among the four protocols (ANOVA, $P<0.05$). The Δ syst BP and Δ diast BP were significantly larger in the ACh and in the Meca treatment protocols than in the sham and in the Atro treatment protocols. The rises in syst BP and diast BP were similar in ACh and Meca treatment protocols. There was no difference between the Atro and sham-treatment protocols. At T+10, there was no difference in Δ syst BP and diast BP between the four protocols.

Table 1 Baseline cardiovascular and hormonal parameters in the sham, acetylcholine (ACh), atropine (Atro) and mecamylamine (Meca) protocols

	Sham $n=6$	*ACh* $n=6$	*Atro+ ACh* $n=6$	*Meca+ ACh* $n=6$
Systolic BP (mmHg)	189±5	188±5	174±15	186±3
Diastolic BP (mmHg)	87±6	82±5	76±6	82±4
Heart rate (min^{-1})	100±7	99±7	103±5	103±9
Noradrenaline (pg ml^{-1})	247±38	267±63	283±61	258±18
Adrenaline (pg ml^{-1})	441±81	328±97	356±96	501±83
Vasopressin (pg ml^{-1})	4±1	2±0	5±2	5±2
Plasma renin activity (ng l^{-1} min^{-1})	130±32	59±22	120±20	73±21
Atrial natriuretic (factor pg ml^{-1})	58±15	40±13	43±11	45±8

Values are expressed as mean ± s.e.mean.

Heart rate At T+2, the mean change in heart rate (Δ HR) was significantly different among the four protocols (Kruskal-Wallis, $P<0.05$). The Δ HR was significantly larger in the ACh and Meca treatment protocols than in the sham and Atro-treatment protocols. Δ HR was similar between ACh and Meca treatment protocols on one hand and between the Atro and sham treatment protocols on the other hand. At T+10, Δ HR was significantly different among the four protocols (ANOVA, $P<0.05$). A significant bradycardia was observed in the ACh, Atro and Meca treatment protocols compared with the sham treatment protocol.

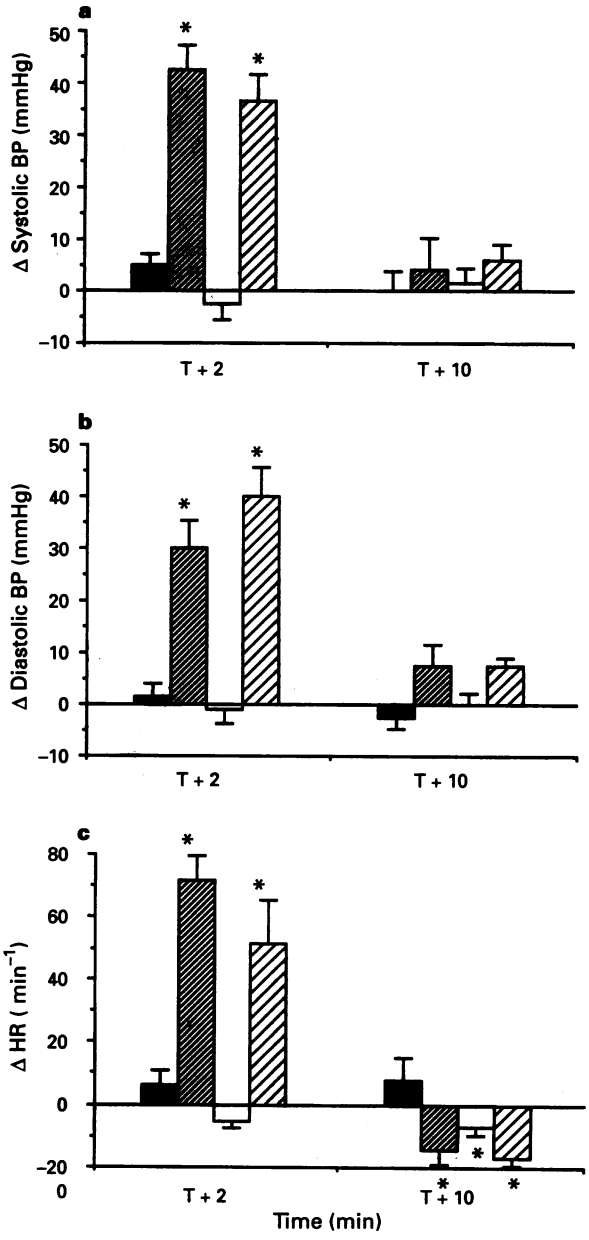

Figure 1 Changes in systolic blood pressure (Δ systolic BP) (a), diastolic blood pressure (Δ diastolic BP) (b) and heart rate (Δ HR) (c) elicited by the sham injection (solid columns) ($n=6$) and by the i.c. injection of 20 μg kg^{-1} of acetylcholine (ACh) in the ACh (closely hatched columns) ($n=6$), atropine (Atro, open columns) ($n=6$) and mecamylamine (Meca, widely hatched columns) ($n=6$) protocols after 2 min (T+2) and 10 min (T+10). In Atro and Meca protocols, an i.c. injection of atropine (5 μg kg^{-1}) and mecamylamine (25 μg kg^{-1}) was performed respectively prior to the i.c. injection of ACh. Values are expressed as variation of the mean ± s.e.mean. At any time, the different protocols are compared to the sham protocol (*$P<0.05$).

Effects of the i.c. injection of ACh on plasma hormonal levels

NA plasma levels (Figure 2a) At T+2, the mean change in NA plasma levels (Δ NA) was significantly different among the four protocols (Kruskal-Wallis, $P<0.05$). Δ NA was significantly larger in the ACh and Meca treatment protocols than in the sham and Atro treatment protocols. Δ NA was similar in ACh and Meca treatment protocols. Δ NA was identical in Atro and sham treatment protocols. At T+10, there was no significant difference in Δ NA between the four protocols.

Ad plasma levels (Figure 2b) At T+2, the mean change in Ad plasma levels (Δ Ad) was significantly different among the four protocols (Kruskal-Wallis, $P<0.05$). Δ Ad was significantly larger in the ACh and Meca treatment protocols than in the sham and Atro treatment protocols. Δ Ad was similar in ACh and Meca treatment protocols. Δ Ad was identical in Atro and Sham treatment protocols. At T+10, there was no significant difference in Δ Ad between the four protocols.

AVP plasma levels (Figure 3a) At T+2, the mean change in AVP plasma levels (Δ AVP) was significantly different among the four protocols (Kruskal-Wallis, $P<0.05$). Δ AVP was significantly larger in the ACh and Meca treatment protocols than in the sham and Atro treatment protocols. Δ AVP was similar in ACh and Meca treatment protocols. Δ AVP was identical in Atro and sham treatment protocols. At T+10, there was no significant difference in Δ AVP between the four protocols.

ANF plasma levels At T+2 and T+10, the mean changes in ANF plasma levels (Δ ANF) were not significantly different among the four protocols.

PRA (Figure 3b) At T+2, the mean change in PRA (Δ PRA) was not significantly different among the four protocols. At T+10, Δ PRA was significantly different among the four protocols (Kruskal-Wallis, $P<0.05$). Δ PRA was reduced in ACh and Meca treatment protocols compared to the sham and Atro treatment protocols. Δ PRA was similar in ACh and Meca treatment protocols. Δ PRA was identical in Atro and sham treatment protocols.

NPY plasma levels The mean basal value of NPY was 403 ± 178 pg ml^{-1} in the ACh treatment protocol. It was not significantly changed at T+2 (402 ± 166 pg ml^{-1}) and T+10 (448 ± 209 pg ml^{-1}).

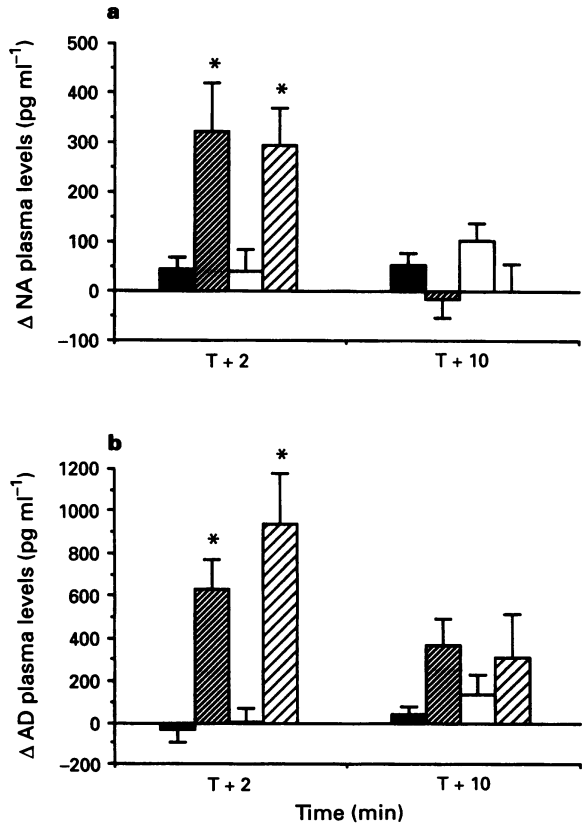

Figure 2 Changes in noradrenaline (Δ NA) (a) and adrenaline (Δ Ad) (b) plasma levels elicited by the sham injection (solid columns) ($n=6$) and by the i.c. injection of 20 μg kg^{-1} of acetylcholine (ACh) in the ACh (closely hatched columns) ($n=6$), atropine (Atro, open columns) ($n=6$) and mecamylamine (Meca, widely hatched columns) ($n=6$) protocols and after 2 min (T+2) and 10 min (T+10). In Atro and Meca protocols, an i.c. injection of atropine (5 μg kg^{-1}) and mecamylamine (25 μg kg^{-1}) was performed, respectively before the i.c. injection of ACh. Values are expressed as variation of the mean ± s.e.mean. At any time, the different protocols are compared to the sham protocol (*$P<0.05$).

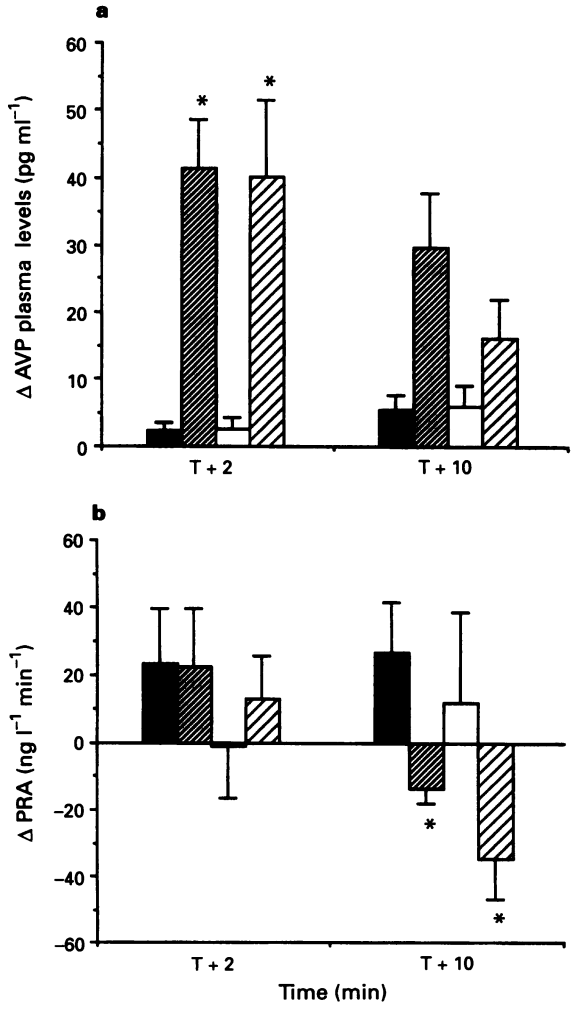

Figure 3 Changes in vasopressin (Δ AVP) plasma levels (a) and plasma renin activity (Δ PRA) (b) elicited by the sham injection (solid columns) ($n=6$) and by the i.c. injection of 20 μg kg^{-1} of acetylcholine (ACh) in the ACh (closely hatched columns) ($n=6$), atropine (Atro, open columns) ($n=6$) and mecamylamine (Meca, widely hatched columns) ($n=6$) protocols and after 2 min (T+2) and 10 min (T+10). In Atro and Meca protocols, an i.c. injection of atropine (5 μg kg^{-1}) and mecamylamine (25 μg kg^{-1}) was performed respectively before the i.c. injection of ACh. Values are expressed as variation of the mean ± s.e.mean. At any time, the different protocols are compared to the sham protocol (*$P<0.05$).

Effects of the i.c. injection of atropine and mecamylamine on cardiovascular responses and hormonal plasma levels (Table 2)

Atropine injection i.c. alone did not itself modify any behavioural, cardiovascular or hormonal plasma parameters. Conversely, mecamylamine injection i.c. induced a transient agitation with tremor associated with a small but significant increase in mean systolic blood pressure (+4 mmHg) (paired-sample Student's t test, $P<0.05$). Moreover, mecamylamine injection i.c. slightly but also significantly increased mean NA plasma levels (+45 pg ml^{-1}) (paired-sample Student's t test, $P<0.05$).

Discussion

We performed the present experiments to investigate the central cardiovascular effects of i.c. ACh in the *conscious* dog. In fact, most of the available studies published on this topic have been performed in anaesthetized animals (for review, see Brezenoff & Giuliano, 1982). In anaesthetized animals, it is generally reported that ACh induces a rise in blood pressure mediated by an increase in both sympathetic tone and the release of AVP (Krstic & Djurcovic, 1978; Buccafusco & Brezenoff, 1979; Rascol *et al.*, 1990). The central effects of cholinomimetic drugs on heart rate and Ad release are on the contrary extremely variable according to the literature. There is substantial evidence that the use of anaesthetics may influence responses. Anaesthetics are known to modify sympathetic and parasympathetic tone (Marty & Reves, 1989). For example, halothane is known to decrease the sympathetic tone (Miller *et al.*, 1983) and ketamine (White *et al.*, 1982) and chloralose (Covert *et al.*, 1992) increase sympathetic tone. Moreover, anaesthetics have significant effects on AVP release (Heller, 1951) and modify the activity of brainstem cholinergic neurones (Bradley & Dray, 1973). Few results are available concerning the central cardiovascular effects mediated by cholinoceptor agonists in conscious animals (cat: Day & Roach, 1977; rat: Buccafusco & Brezenoff, 1979; Hoffmann, 1979). We are aware of only one study in conscious dogs (Lang & Rush, 1973). We therefore reassessed the role of cholinergic systems in a conscious dog model with a chronically implanted intracisternal cannula that allows repeated central i.c. injections of different cholinoceptor agents.

With this procedure, we have demonstrated in the conscious dog that i.c. injection of ACh induces: (1) a rise in systolic and diastolic blood pressures, (2) a transient tachycardia followed by a long lasting bradycardia, (3) an increase in NA, Ad and AVP plasma levels, (4) and that atropine but not mecamylamine completely blocks all of these cardiovascular and hormonal responses.

Cardiovascular responses

The present data demonstrate that ACh (20 $\mu g\ kg^{-1}$, i.c.) induces a pressor response in conscious animals. This effect is qualitatively comparable in magnitude to what was previously observed with a similar protocol in anaesthetized dogs (Rascol *et al.*, 1990). The duration of the response was however nearly twice as long in the chloralose-anaesthetized dog (Rascol *et al.*, 1990). A comparable rise in blood pressure after central injection of cholinomimetic drugs has also been reported in anaesthetized and conscious rat (Xiao & Brezenoff, 1988; Buccafusco & Brezenoff, 1979). Conversely, conflicting results have been reported in the cat with a cholinergic pressor effect in the conscious cat (Day & Roach, 1977) and a depressor effect in the anaesthetized cat (Ally *et al.*, 1992). It is possible that anaesthesia may qualitatively modify the cholinergic pressor response in the cat but not in other species.

In contrast to blood pressure, the changes in heart rate that we observed in the conscious dogs were markedly different from those that we had previously reported in the chloralosed-anaesthetized dogs (Rascol *et al.*, 1990). In anaesthetized dogs, we detected no significant change in heart rate. In the literature, results in anaesthetized animals are quite variable: some authors reported that central ACh induces tachycardia (Krstic & Djurkovic, 1978; Hoffman, 1979), while others observed bradycardia (Pazos *et al.*, 1986; Xiao & Brezenoff, 1988) or no significant change at all (Buccafusco & Brezenoff, 1979). These discrepancies are probably explained by the fact that heart rate is closely dependent on the level of anaesthesia. In the present study, i.c. ACh induced a clear biphasic heart rate response: a transient initial tachycardia followed by bradycardia.

The initial tachycardia often was associated with a transient behavioural change of increased alertness, licking and swallowing. This behaviour was not related to a non specific or painful effect of the i.c. injection because it was not observed in the sham group and was abolished by atropine. A similar central ACh effect on behaviour has been reported by other authors (Lang & Rush, 1973; Day & Roach, 1977; Buccafusco & Brezenoff, 1979). It has been related to a concomitant increase in catecholamine plasma levels (Kennedy *et al.*, 1984) which was also observed in our experiments. However, it must be noted that the timing of the behavioural changes (lasting less than 1 min) was different from that of the rise in catecholamine plasma levels (exceeding 2 min). It is also possible that the observed tachycardia did not reflect only a sympathetic response but was related to a direct effect of ACh on specific brainstem cardiac centres. In fact, cholinoceptors have been localized in areas involved in heart rate regulation such as the dorsal vagal complex and the nucleus tractus solitari (Hyde *et al.*, 1988).

The bradycardia that followed the initial transient tachycardia can be explained in two different ways. The first involves the baroreflex. It is however surprising that if the bradycardia was of reflex origin it persisted for longer than 10 min while blood pressure, NA, Ad and AVP plasma levels had already returned to basal values. It has been suggested that central injections of cholinomimetic agents can modify the baroreflex response (Caputi *et al.*, 1980). It is thus possible that the bradycardia observed in our experiment was indeed of baroreflex origin. A second explanation could be that the bradycardia was indirectly related to a central sedative effect of i.c. ACh. We frequently noticed that the transient period of alertness was followed by a period of quietness and drowsiness.

Table 2 Cardiovascular and hormonal parameters 1 min before and 5 min after ic injection of atropine (5 $\mu g\ kg^{-1}$) or mecamylamine (25 $\mu g\ kg^{-1}$)

	Injection of atropine $n=6$		*Injection of mecamylamine* $n=6$	
	before	*after*	*before*	*after*
Systolic BP (mmHg)	184±7	174±16	182±2	186±3*
Diastolic BP (mmHg)	83±5	76±6	78±4	82±4
HR (min^{-1})	104±6	103±5	108±11	103±9
Noradrenaline (pg ml^{-1})	328±79	283±60	203±18	258±18*
Adrenaline (pg ml^{-1})	313±135	356±96	371±60	501±83
Vasopressin (pg ml^{-1})	1±0	5±2	3±1	5±2
Plasma renin activity (ng l^{-1} min^{-1})	84±8	120±20	59±19	73±21
Atrial natriuretic (factor pg ml^{-1})	42±12	43±11	52±11	45±8

Values are expressed as mean ± s.e.mean. * $P<0.05$.before vs after.

This second period lasted several minutes. This behaviour has also been reported by others in the dog (Lang & Rush, 1973) and in the rat (Buccafusco & Brezenoff, 1979). In fact, the central cholinergic systems have been implicated in sleep mechanisms (Gillin *et al.*, 1985) and bradycardia frequently accompanies sleep (Van De Borne *et al.*, 1994).

Hormonal plasma levels

The i.c. injection of ACh induced rises in plasma NA and AVP that were similar in magnitude to those reported in chloralose-anaesthetized dogs (Rascol *et al.*, 1990). The duration of the hormonal changes was however shorter in the conscious animals as already noted for the pressor response in the same animals. The similar timing of the blood pressure and hormonal changes in conscious and anaesthetized animals suggests that the rise in blood pressure is produced by the peripheral vasoconstrictor properties of the hormones. AVP plasma levels rose to 40–45 pg ml^{-1}, a magnitude compatible with the physiological vasoconstrictor properties of AVP in the conscious dog (Rossi & Schrier, 1986). Anaesthesia had no significant effect on the dynamics of the AVP increase but, the mean basal values of AVP observed in the conscious dogs (4 pg ml^{-1}) were smaller than those that we had previously reported in the anaesthetized animals (13 pg ml^{-1}) (Heller, 1951).

The i.c. injection of ACh also produced a significant rise in Ad plasma levels. This is in contrast with what we previously observed in the anaesthetized dog (Rascol *et al.*, 1990). The role of Ad in mediating the central cholinergic pressor response has been frequently debated. Adrenalectomy in the rat reduces the rise in blood pressure induced by i.c. ACh (Brezenoff, 1973; Krstic & Djurkovic, 1978). However, this result has not been unequivocally reported (Buccafusco & Brezenoff, 1979). In conscious man, Kennedy *et al.* (1984) found that i.v. physostigmine significantly increased Ad plasma levels.

The i.c. ACh injection did not modify NPY plasma levels. NPY is a peptide colocated with NA in cardiovascular sympathetic nerves (Lundberg *et al.*, 1990). We hypothesized that NPY could have been co-liberated with NA in our experiment. However, we observed no change in NPY. This result is in fact in agreement with other data suggesting that there is a threshold for NPY co-liberation. This threshold has been estimated as a seven times increase in NA plasma levels (Poncet *et al.*, 1992) and was not reached in our protocol (two times increase in NA plasma levels).

It is well known that sympathetic stimulation induces renin release (Vander, 1965). We anticipated that the increase of the sympathetic tone induced by i.c. ACh would have increased PRA in our experiments as previously described with physostigmine in the rat by Alexandre *et al.* (1970). In contrast, we observed that PRA was slightly but significantly reduced 10 min after the i.c. injection of ACh. This phenomenon was not observed in anaesthetized animals (Rascol *et al.*, 1990) but has been reported in conscious rats (Kawashima *et al.*, 1987; Verma & Taylor, 1990). In fact, the secretion of renin may be delayed compared to that of other hormones like AVP (Guyton, 1986). Since AVP inhibits PRA (Vander, 1968) we suggest that the expected increase in PRA to the increased sympathetic tone was masked by the opposite effect of the large and rapid release of AVP.

We were surprised to notice that ANF plasma levels were not significantly modified by the i.c. injection of ACh in conscious dogs. In fact, several changes induced by i.c. ACh (rise in blood pressure, NA and AVP plasma levels) are known to increase ANF plasma levels (Manning *et al.*, 1985). We have observed an increase in ANF plasma levels after the central injection of ACh in our past experiments in anaesthetized dogs (Rascol *et al.*, 1990). A similar result was reported in the conscious rat (Massi *et al.*, 1991). We have no clear explanation for the lack of an increase in ANF in the present experiments.

Effects of cholinoceptor antagonists on i.c. ACh responses

All the significant changes in measured parameters that were induced by i.c. ACh in the conscious dogs were blocked by i.c. atropine. This observation demonstrates that central muscarinic receptors are mediating these effects. This is in good agreement with several studies conducted in anaesthetized animals (Sinha *et al.*, 1967; Krstic & Djurkovic, 1978; Buccafusco & Brezenoff, 1979; Hoffman, 1979). Some authors however have suggested that nicotinic receptors are also involved in some aspects of these responses. This is true for example for AVP release (Milton & Paterson, 1974; Sladeck & Joynt, 1979). Our results do not confirm these data since i.c. injection of mecamylamine did not block any cholinoceptor mediated cardiovascular or hormonal responses including AVP release.

The lack of effect of i.c. atropine on baseline cardiovascular and hormonal parameters suggests that there is little or no central muscarinic cholinergic tone controlling the cardiovascular and hormonal parameters that we have measured. We observed however that 10 min after i.c. atropine heart rate was slightly but significantly reduced compared to baseline (see Figure 1). It is unlikely that this bradycardia was due to an insufficient blockade of muscarinic receptors because all the other cholinoceptor responses were fully blocked. We alternatively suggest that the bradycardia could be related to a direct effect of i.c. atropine. In fact, atropine is known to induce a bradycardia through a central vagal stimulation (Weiner, 1985).

Central injection of mecamylamine produced a moderate but significant rise in blood pressure and plasma NA levels. Since nicotine induces cholinoceptor agonist effects before blocking the nicotinic receptors (Ginsborg & Guerrero, 1964) a similar explanation can be suggested for mecamylamine but a non specific effect of the drug cannot be excluded.

We suggest that the observed cardiovascular effects of ACh could be localized in medulla areas because of the proximity of the cisterna magna and caudal medulla. These medullary areas are known to be involved in the physiological regulation of cardiovascular functions (Chalmers & Pilowsky, 1991) and to contain muscarinic receptors (Hyde *et al.*, 1988; Sudaram *et al.*, 1988). It is not possible however to exclude other possibilities. Considering the fact that ACh can dilate blood vessels with which it comes into contact, this phenomenon could result in compensatory changes in sympathetic outflow. It must also be noticed that we cannot definitely rule out the possibility that ACh acting via muscarinic receptors had effects primarily directed to neural pathways involving the behavioural changes observed. The cardiovascular and neurohormonal responses may then have been secondary to the behavioural responses elicited by ACh. However, this hypothesis seems unlikely for two reasons. First, behavioural changes were not observed in all dogs although the cardiovascular and neurohormonal responses were always present. Second, the same type of responses was observed in the anaesthetized dogs where behavioural changes were avoided (Rascol *et al.*, 1990).

In conclusion, the present data suggest that the i.c. effects of ACh observed in the conscious dogs differ from those reported in anaesthetized dogs. In fact, the changes in blood pressure, NA and plasma AVP levels in the conscious dog have a similar amplitude but a shorter duration than those previously reported in anaesthetized dogs. The central cholinoceptor response of heart rate and Ad plasma levels are more markedly modified by anaesthesia. We observed a biphasic heart rate response (short term tachycardia/long lasting bradycardia) and the release of Ad in conscious animals while these responses had not been previously detected under chloralose anaesthesia. We conclude that in the conscious dog the cardiovascular changes induced by the central injection of ACh can be related to an increase in sympathetic outflow and in AVP release while plasma ANF, NPY

and PRA seem to have little influence. Moreover, these cardiovascular and hormonal responses are mediated by the stimulation of central muscarinic receptors.

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