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Characterization of the central muscarinic cholinoceptors involved in the cholinergic pressor response in anesthetized dogs

Michel Pelat [a], Eric Lazartigues [a], Marie-Antoinette Tran [a], Claude Gharib [b], Jean-Louis Montastruc [a], Paul Montastruc [a], Olivier Rascol [a,*]

[a] *Laboratoire de Pharmacologie Médicale et Clinique, INSERM U317 et U455, Faculté de Médecine, 37 Allées Jules Guesde 31073, Toulouse, Cedex, France*

[b] *Laboratoire de Physiologie de l'environment, Faculté de Médecine de Lyon, Lyon, France*

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Abstract

Previous reports have shown that an intracisternal (i.c.) injection of acetylcholine in the dog increases both arterial blood pressure and plasma levels of noradrenaline and vasopressin via central muscarinic receptors. The aim of the present study was to characterize the central muscarinic cholinoceptor subtypes involved in such central cholinergic responses in anesthetized male Beagle–Harrier dogs ($n = 12$). For this purpose, we studied the relative potency of various muscarinic receptor antagonists to block the acetylcholine-induced pressor responses (30 $\mu g\ kg^{-1}$ i.c.). The acetylcholine-induced pressor response was inhibited in a dose-dependent manner by the i.c. administration of the non-selective muscarinic receptor antagonist atropine ($ID_{50} = 0.5\ \mu g\ kg^{-1}$), the muscarinic M_1 receptor antagonist pirenzepine ($ID_{50} = 0.45\ \mu g\ kg^{-1}$), the muscarinic M_2 receptor antagonist methoctramine ($ID_{50} = 8.5\ \mu g\ kg^{-1}$) and the muscarinic M_3 receptor antagonist *para*-fluoro-hexahydro-sila-difenidol ($ID_{50} = 43.7\ \mu g\ kg^{-1}$). The order of potency of these four muscarinic receptor antagonists was: atropine = pirenzepine > methoctramine ≫ *para*-fluoro-hexahydro-sila-difenidol. In order to confirm the selectivity for muscarinic M_1 receptors of this dose of pirenzepine, we checked that 40- to 50-fold higher concentrations were necessary to block a typical muscarinic M_2 receptor response (bradycardia) and a typical muscarinic M_3 receptor response (endothelial vasodilation) compared with methoctramine and *para*-fluoro-hexahydro-sila-difenidol, respectively. These results suggest that the pressor response elicited by intracisternal injection of acetylcholine in anesthetized Beagle–Harrier dogs is mediated through the activation of the muscarinic M_1 cholinoceptor subtype.

Keywords: Acetylcholine; Muscarinic receptor; Blood pressure; Pirenzepine

1. Introduction

The first in-depth study of central cholinergic involvement in cardiovascular regulation was reported by Suh et al. (1935). Since then, it is well known that central muscarinic cholinoceptors are involved in the regulation of blood pressure and heart rate (Brezenoff and Giuliano, 1982; Buccafusco, 1996). Stimulation of these receptors by direct or indirect cholinomimetic agents results in marked pressor responses primarily mediated via an increase in sympathetic tone (Krstic and Djurkovic, 1978; Buccafusco and Brezenoff, 1979; Brezenoff and Giuliano, 1982; Buccafusco, 1996). The cDNAs of five different muscarinic cholinoceptor subtypes have been cloned (Bonner et al., 1987). Currently, at least three different functional muscarinic cholinoceptor subtypes (M_1, M_2, M_3) have been defined on the basis of pharmacological studies using selective antagonists (Caulfield, 1993). In addition, there is a candidate functional muscarinic M_4 cholinoceptor found in the striatum (Waelbroeck et al., 1990), some cell lines (e.g., N.G108-15) (Michel et al., 1989; Lazareno et al., 1990), chicken atria (Tietje et al., 1990), rabbit lung (Lazareno et al., 1990), guinea-pig gallbladder (Kurtel et al., 1990), and uterus (Dorje et al., 1990; Özkutlu et al., 1993; Eglen and Watson, 1996). A physiological role for the *m5* gene product remains to be identified (Hosey, 1992).

* Corresponding author. Tel.: +33-5-61-52-98-44; fax: +33-5-61-25-51-16; E-mail: rascol@cict.fr

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On one hand, previous reports suggest that the central cholinergic cardiovascular effects of physostigmine in rats (Brezenoff et al., 1988; Lazartigues et al., 1998a,b) and anesthetized cats (Ally et al., 1995) are possibly mediated through an activation of the muscarinic M_2 cholinoceptor subtype in the central nervous system. On another hand, Hori et al. (1995) reported a muscarinic M_1 receptor-mediated increase in blood pressure following intrahippocampal neostigmine injection in rat. To our best knowledge, the characterization of the muscarinic cholinoceptor subtype(s) involved in the pressor response to i.c. acetylcholine has not yet been performed in dogs.

The aim of this study was to identify which subtype(s) of muscarinic cholinoceptor(s) is (are) involved in the central cardiovascular responses elicited by acetylcholine in anesthetized dogs. For this purpose, the effects of the non-selective muscarinic cholinoceptor antagonist, atropine and those of the partially selective muscarinic cholinoceptor antagonists pirenzepine, (a muscarinic M_1 receptor antagonist), methoctramine (a muscarinic M_2 receptor antagonist) and p-F-HHSiD (a muscarinic M_3 receptor antagonist) were investigated on acetylcholine-induced pressor response.

2. Materials and methods

2.1. General protocol

Experiments were performed on two groups of six anesthetized Beagle–Harrier male dogs (D'Ambrieres, Aze, France) weighing 10 to 15 kg. The dogs were fasted on the morning of the experiment but had free access to water ad libitum in order to be normally hydrated. All animals procedures were conducted in strict compliance with approved French Agriculture Department for Animal Use for Research and Education protocols.

Animals were anesthetized with α-chloralose (100 mg kg^{-1}, i.v.), intubated and ventilated with an Ideal Palmer respirator (insufflated air volume: 15 ml kg^{-1} with a frequency of 16 min^{-1}).

The degree of anesthesia was evaluated by the lack of eyelid reflex, the lack of reactions to any external stimulus and the stability of cardiovascular parameters. Body temperature of the animal was maintained at a constant level of 38°C. A catheter was introduced into the left femoral artery at the beginning of the experiment. It was connected to a Statham P23Db transducer to monitor systolic and diastolic blood pressure and was removed at the end of each experiment. Heart rate was counted on the electrocardiogram (lead II). These parameters were continuously recorded on a Beckman recorder (type R411). A cannula was also inserted into the cisterna magna (between C1 and the skull). The proper position of the cannula within the cisterna magna was demonstrated by the flow of cerebrospinal fluid into the cannula.

The collection of experimental data started 10 min after the insertion of the intracisternal (i.c.) cannula to allow cardiovascular parameters to return to basal values after this potentially stressful event.

Blood samples for catecholamines and vasopressin plasma levels assays were obtained from the femoral artery. The volume of blood removed for plasma assay was immediately replaced by an equivalent volume (10 ml) of isotonic saline.

2.2. Cardiovascular and hormonal effects of i.c. acetylcholine in the dog

On the first group of dogs ($n = 6$), we assessed the cardiovascular and hormonal effects of a standard dose of acetylcholine (30 μg kg^{-1}, i.c., $n = 6$), in comparison with those of a saline i.c. injection (0.3 ml, $n = 6$). The inhibition of this pressor response was also verified following pretreatment with atropine (0.5–2 μg kg^{-1}, i.c., $n = 6$) as previously reported by Brefel et al. (1995). These parameters were measured at five different times: 10 and 5 min before (T-10 and T-5), then 5, 15 and 25 min (T5, T15 and T25) after i.c. injection.

2.3. Effects of selective muscarinic cholinoceptors antagonists

In order to determine the subtype(s) of central muscarinic cholinoceptor(s) involved in the acetylcholine-induced pressor response (30 μg kg^{-1}), we randomly administered in a second group of dogs ($n = 6$), various muscarinic receptor antagonists by i.c. route, 10 min prior to the standard acetylcholine i.c. injection. At least three doses of each antagonist (pirenzepine: 0.5–20 μg kg^{-1}, i.c.; methoctramine: 1–20 μg kg^{-1}, i.c.; and *para*-fluorohexahydro-sila-difenidol (p-F-HHSiD): 10–50 μg kg^{-1}, i.c.) were tested in at least five experiments. For each point, five or fewer experiments were performed in the same dog.

A 10-day wash-out interval was respected between each i.c. injection.

In order to confirm that the muscarinic receptor antagonists remained selectives at the dose used, we assessed in six conscious dogs, their relative potencies on two well known cholinergic responses: first, the tachycardia elicited by blockade of muscarinic M_2 cardiac receptors (Van Zwieten et al., 1995) and secondly, the decrease in blood pressure measured as a consequence of vasodilation induced by the stimulation of muscarinic M_3 endothelial receptors (Boulanger et al., 1994).

2.4. Measurement of catecholamines and vasopressin plasma levels

All measurements were done blind. For catecholamines plasma levels determination, blood was collected in

lithium-heparin tubes. For noradrenaline, the tubes contained 10 mM of sodium metabisulphite. Blood samples were then centrifuged at $4000 \times g$ for 15 min at 0°C. Plasma was stored at −80°C. Noradrenaline and vasopressin levels were measured as previously described by Brefel et al. (1995).

2.5. Drugs

The following drugs were used: α-chloralose (Prolabo, Paris, France), atropine sulfate (a non-selective muscarinic receptor antagonist), pirenzepine (Sigma, St. Quentin Fallavier, France), methoctramine and p-F-HHSiD (RBI, Natick, USA). All the drugs were dissolved in physiological saline excepted for p-F-HHSiD which was dissolved in 10% ethanol (the vehicle was tested to make sure that it did not induce, by itself, any effect on blood pressure and heart rate).

2.6. Statistical analysis

According to the homogeneity of variances, a one-way analysis of variance (ANOVA), followed by a Scheffe's post-hoc test, was performed to compare baseline values of the different parameters, in each protocol, in order to assess if there was any significant intergroup difference.

A paired-sample Student's *t*-test was used to compare the different parameters, before and after pretreatment by the i.c. injection of saline or the muscarinic receptor antagonists, in order to assess if this pretreatment induced any effects by itself. An ANOVA for repeated measures, followed by a Dunnett's post-hoc test was used to compare the mean variations (Δ) of the different parameters at the different times, in each treatment group, in order to assess the acetylcholine-induced pressor response. Dose–response curves were fitted by a non-linear regression to a sigmoïdal and ID_{50} calculated using the program Prism (GraphPad Software, San Diego, CA, USA). Values are expressed as means ± S.E.M. The level of significance was accepted for $P < 0.05$.

3. Results

The baseline values for cardiovascular and hormonal parameters were not significantly different before i.c. injections in each group of dogs (Table 1). Saline induced no significant change in any parameter following i.c. injection at any time (data not shown).

3.1. Effects of the i.c. injection of acetylcholine on cardiovascular and hormonal responses

The i.c. injection of acetylcholine induced, within 5 min, a significant increase in systolic and diastolic blood pressure ($+38 \pm 7$ mmHg, $+28 \pm 4$ mmHg, respectively, ANOVA $P < 0.05$) and remained increased up to 15 min after administration, until values returned progressively to baseline within 25 min (Fig. 1A).

Noradrenaline and vasopressin plasma levels were also significantly modified by acetylcholine (Fig. 1B). At T5 and T15 min, following acetylcholine administration, noradrenaline plasma levels were significantly increased ($+275 \pm 41$ pg ml^{-1}, $+152 \pm 5$ pg ml^{-1}, respectively, ANOVA $P < 0.05$). Conversely, vasopressin plasma levels were only significantly increased at T15 min.

3.2. Effects of the muscarinic receptor antagonists

The different antagonists used in the study induced no significant change by themselves on any measured parameters, excepted for methoctramine. Indeed, the highest dose (20 μg kg^{-1}) of this muscarinic M_2 receptor antagonist induced a significant increase in both systolic and diastolic blood pressure and vasopressin plasma levels ($+21 \pm 3$ and $+13 \pm 3$ mmHg and $+22 \pm 10$ pg ml^{-1}, respectively, $P < 0.05$). The same dose of this muscarinic M_2 receptor antagonist, induced no significant change in noradrenaline plasma levels.

3.3. Effects of the different muscarinic receptor antagonists on the acetylcholine-induced pressor and hormonal responses

The four muscarinic antagonists blocked in a dose-dependent way the increase in blood pressure evoked by acetylcholine.

Pretreatment of the dogs by an i.c. injection of three doses: 0.5, 1, 2 μg kg^{-1} of atropine resulted in a dose-dependent inhibition of the peak acetylcholine (30 μg kg^{-1}) effects on blood pressure ($P < 0.05$). Dose–response curves analysis showed that atropine had an ID_{50} equal to 0.5 μg kg^{-1} (Fig. 2).

Table 1
Baseline cardiovascular and hormonal parameters in the five protocols. Values are expressed as mean ± S.E.M.

	Group 1		Group 2			ANOVA
	Saline ($n = 6$)	Atropine ($n = 6$)	Pirenzepine ($n = 6$)	Methoctramine ($n = 6$)	pFHHSiD ($n = 6$)	
Systolic blood pressure (mmHg)	203 ± 9	193 ± 8	197 ± 9	208 ± 4	201 ± 3	NS
Diastolic blood pressure (mmHg)	109 ± 6	106 ± 7	107 ± 4	118 ± 3	110 ± 3	NS
Noradrenaline plasma levels (pg ml^{-1})	195 ± 43	140 ± 26	230 ± 72	152 ± 23	121 ± 23	NS
Vasopressin plasma levels (pg ml^{-1})	2 ± 1	1 ± 0	2 ± 1	2 ± 1	1 ± 0	NS

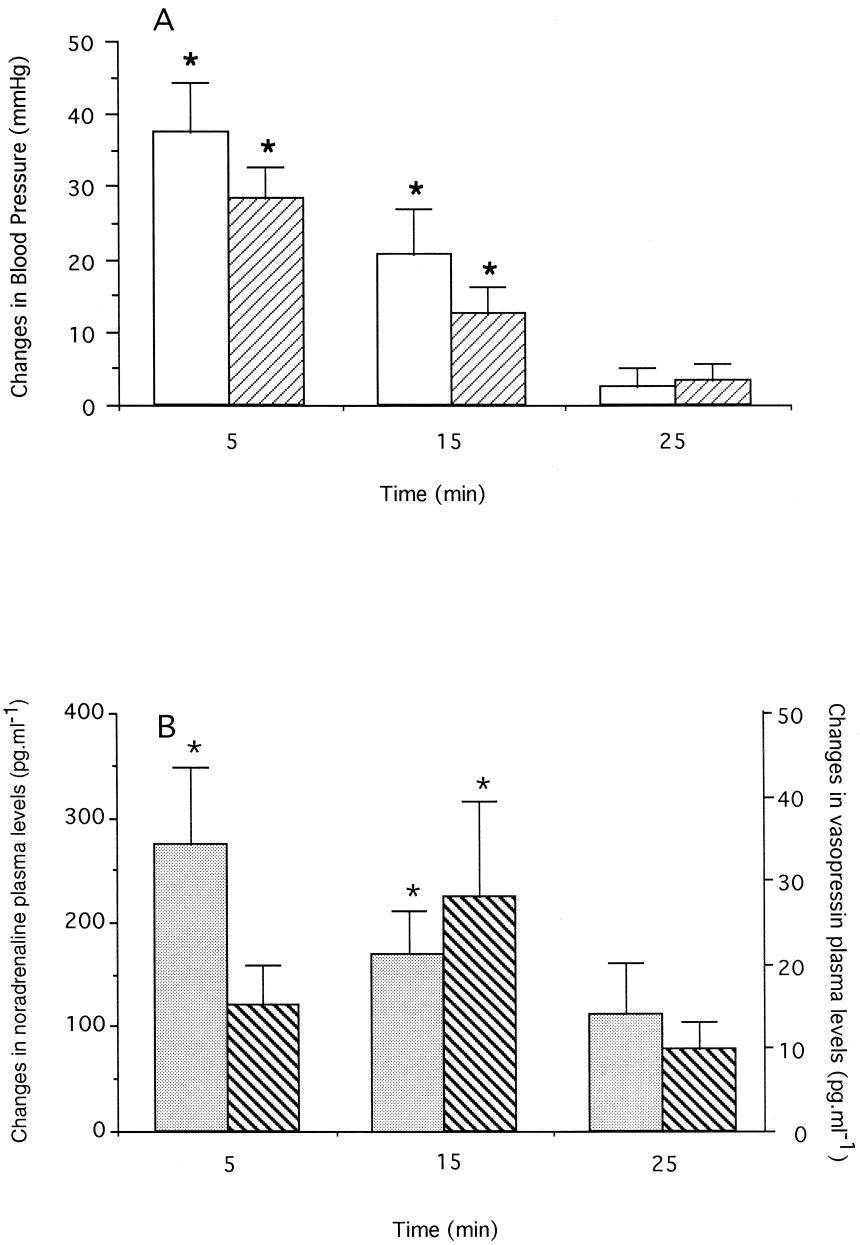

Fig. 1. Changes in systolic (open columns) and diastolic (hatched columns) blood pressure (A) and noradrenaline (shaded columns) and vasopressin (hatched columns) plasma levels (B) elicited by the i.c. injection of 30 μg kg^{-1} of acetylcholine. Values are expressed as mean variations ± S.E.M. Statistical significance vs. basal values: $^{*}P < 0.05$.

Pretreatment with pirenzepine (0.5–20 μg kg^{-1} i.c.) inhibited in a dose-dependent way the acetylcholine-induced blood pressure response. The ID_{50} calculated from the dose–response curves was equal to 0.45 μg kg^{-1} ($P < 0.05$, Fig. 2).

Methoctramine pretreatment (1–20 μg kg^{-1} i.c.) inhibited in a dose-dependent way the increase in blood pressure evoked by 30 μg kg^{-1} i.v. of acetylcholine with an ID_{50} equal to 8.5 μg kg^{-1} ($P < 0.05$, Fig. 2).

The acetylcholine-induced pressor response was also dose-dependently inhibited by p-F-HHSiD (10–50 μg kg^{-1} i.c.), the ID_{50} being equal to 43.7 μg kg^{-1} ($P < 0.05$, Fig. 2).

The potency order for the participation of muscarinic receptors in the pressor response to i.c. acetylcholine was then: atropine = $M_1 > M_2 \gg M_3$.

Moreover, Figs. 2 and 3 show the changes in systolic and diastolic pressure and in hormonal plasma levels, elicited by i.c. acetylcholine injection following i.c. pretreatment by the minimal dose for each antagonist which blocked the cholinergic pressor effect. At T5 and T15, both systolic and diastolic blood pressure and noradrenaline plasma levels were significantly increased in the acetylcholine group in comparison with the four other groups. ($P < 0.05$) (Figs. 2 and 3A). Atropine (1 μg kg^{-1}), pirenzepine (1 μg kg^{-1}), methoctramine (20 μg

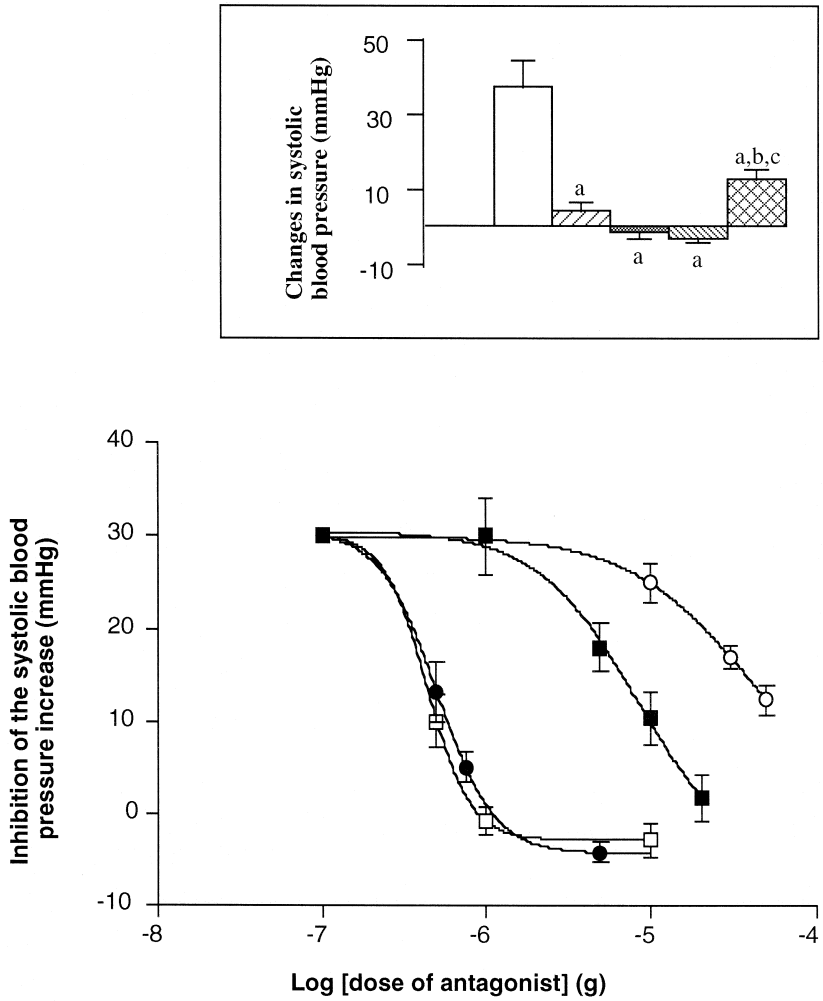

Fig. 2. The pressor response induced by i.c. acetylcholine (30 μg kg^{-1}) was inhibited by appropriate amounts of the no selective muscarinic receptor antagonist atropine (●) (5–2 μg kg^{-1}) or the selective muscarinic receptor antagonists pirenzepine (□) (M_1 antagonist, 0.5–10 μg kg^{-1}), methoctramine (■) (M_2 antagonist, 1–20 μg kg^{-1}) and *para*-fluoro-hexahydro-sila-difenidol (○) (M_3 antagonist, 10–50 μg kg^{-1}). All points consist of six experiments. Values are expressed as means ± S.E.M. Insert: changes in systolic pressure elicited 5 min after i.c. acetylcholine injection (30 μg kg^{-1}) following i.c. pretreatment by saline (0.3 ml, open columns), atropine (1 μg kg^{-1} widely hatched columns), pirenzepine (1 μg kg^{-1}, shaded columns), methoctramine (20 μg kg^{-1}, closely hatched columns) and *para*-fluoro-hexahydro-sila-difenidol (50 μg kg^{-1}, double hatched columns). Each group consist of six animals. Values are expressed as means ± S.E.M. Statistical significance vs. acetylcholine (a), pirenzepine (b), and methoctramine (c) groups: $P < 0.05$.

kg^{-1}) and p-F-HHSiD (50 μg kg^{-1}) significantly reduced the increase in blood pressure and the noradrenaline plasma level increase evoked by acetylcholine (Figs. 2 and 3A).

Furthermore, at T5, atropine (1 μg kg^{-1}), pirenzepine (1 μg kg^{-1}), p-F-HHSiD (50 μg kg^{-1}), but not methoctramine, significantly reduced the increase in vasopressin plasma levels evoked by acetylcholine (Fig. 3B). At T15, there was no difference in the vasopressin plasma levels among the five protocols (ANOVA $P = 0.07$) (Fig. 3B).

3.4. Effects of the muscarinic receptor antagonists on characterized peripheral cholinergic effects

A dose of 50 μg kg^{-1} i.v. of atropine and methoctramine was sufficient to increase heart rate by $+119 \pm 13$ and $+114 \pm 14$ bpm, respectively ($n = 6$). Conversely, 2 mg kg^{-1} i.v of pirenzepine was necessary to induce a similar tachycardia ($+108 \pm 12$ bpm, $n = 6$).

The i.v. injection of 2 μg kg^{-1} of acetylcholine decreased blood pressure by 50 ± 5 mmHg in dogs. This endothelium-dependent vasodilation, known to be mediated by muscarinic M_3 receptors, was antagonized by a pretreatment of 200 μg kg^{-1} i.v. of atropine ($n = 6$) or 800 μg kg^{-1} i.v. of p-F-HHSiD ($n = 4$). Conversely, a higher dose of pirenzepine (40 to 50-fold higher than atropine) was necessary to block this effect.

4. Discussion

We performed the present study to characterize the muscarinic cholinoceptor subtype(s) involved in the central cardiovascular effects of i.c. acetylcholine in anesthetized

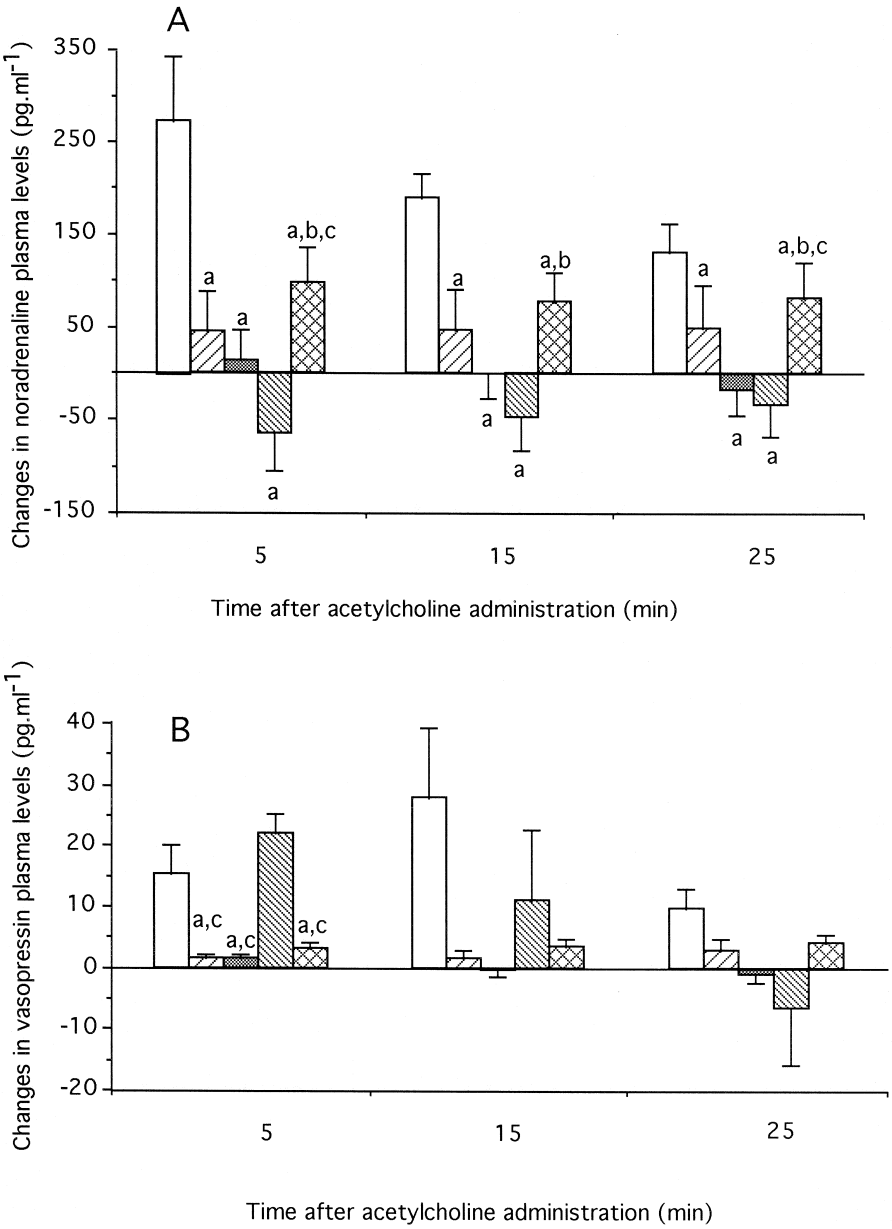

Fig. 3. Changes in noradrenaline (A) and vasopressin (B) plasma levels are elicited by i.c. acetylcholine injection (30 μg kg^{-1}) following i.c. pretreatment by saline (0.3 ml, open columns) atropine (1 μg kg^{-1}, widely hatched columns), pirenzepine (1 μg kg^{-1}, shaded columns), methoctramine (20 μg kg^{-1}, closely hatched columns) and *para*-fluoro-hexahydro-sila-difenidol (50 μg kg^{-1}, double hatched columns). Each group consist of six animals. Values are expressed as means ± S.E.M. Statistical significance vs. acetylcholine (a), pirenzepine (b), and methoctramine (c) groups: $P < 0.05$.

dogs. In fact, most of the available studies published on this topic have been performed in two other species: rats and cats. The central pressor response elicited by the administration of cholinoceptor agents (acetylcholine, cholinesterase inhibitors, cholinoceptor agonists) is known to involve muscarinic cholinoceptors, since this pressor response is blocked by atropine pretreatment (Brezenoff and Giuliano, 1982; Buccafusco, 1996). Previous reports from our laboratory have confirmed that a pretreatment with the non-selective muscarinic cholinoceptor antagonist atropine also abolishes such acetylcholine-induced cardiovascular responses in the dog (Brefel et al., 1995). However, the subtypes of muscarinic cholinoceptors involved in this central cholinergic pressor response remains a matter of controversy according to the literature. For example, it has been suggested that the muscarinic M_1 cholinoceptor subtype plays a role in the regulation of cardiovascular responses in spontaneous hypertensive rats (Wei et al., 1995). It has also been reported that the muscarinic M_2 cholinoceptor subtype is involved in the cardiovascular responses elicited by a cholinergic stimulation in normotensive rats (Özkutlu et al., 1993; Lazartigues et al., 1998a,b) as well as in cats (Ally et al., 1995). Martin (1992) reported that the muscarinic M_3 cholinoceptor sub-

type might also be involved in the cholinergic central pressor response observed in the rat. To our best knowledge, there has been no study identifying the subtype of muscarinic cholinoceptors involved in the central cardiovascular effects of acetylcholine in the dog.

The present results demonstrate as expected from previous experiments (Rascol et al., 1990; Brefel et al., 1995) that i.c. administration of acetylcholine (30 $\mu g\ kg^{-1}$) evokes a significant increase in both blood pressure and noradrenaline and vasopressin plasma levels. These effects are mediated through the stimulation of central muscarinic cholinoceptors, since a central pretreatment with the non-''selective'' muscarinic receptor antagonist, atropine (1 $\mu g\ kg^{-1}$) completely blocks them. To our best knowledge, there are no studies in dogs assessing the effect of the i.c. administration of either pirenzepine, methoctramine or p-F-HHSiD on such effects. Pirenzepine is widely used as a selective antagonist for the muscarinic M_1 cholinoceptors ($pA_2 = 8.3$) (Martin, 1992; Eglen and Watson, 1996). Binding studies have demonstrated that methoctramine is a ''selective'' muscarinic M_2 cholinoceptor blocker ($pA_2 = 7.9$) and that p-F-HHSiD is a ''selective'' antagonist for the muscarinic M_3 cholinoceptors ($pA_2 = 7.9$) (Eglen et al., 1990; Martin, 1992; Eglen and Watson, 1996).

We assessed the ability of each antagonist to block in a dose-dependent way the acetylcholine-induced pressor response. The ID_{50} values were 0.5 $\mu g\ kg^{-1}$, 0.45 $\mu g\ kg^{-1}$, 8.5 $\mu g\ kg^{-1}$, 43.5 $\mu g\ kg^{-1}$, respectively, for atropine, pirenzepine, methoctramine and p-F-HHSiD. Moreover, our results show that atropine (1 $\mu g\ kg^{-1}$ i.c)., pirenzepine (1 $\mu g\ kg^{-1}$ i.c.) and methoctramine (20 $\mu g\ kg^{-1}$ i.c.) completely abolished these pressor and hormonal effects. The muscarinic M_3 cholinoceptor antagonist p-F-HHSiD, even at the dose of 50 $\mu g\ kg^{-1}$ i.c., did not block completely the acetylcholine-induced pressor response. So, for this compound a higher dose was necessary to completely block this pressor effect.

In our study, and according to the ID_{50} data, the order of potency between each antagonist on this pressor response was atropine = pirenzepine > methoctramine ≫ p-F-HHSiD, suggesting that M_1 cholinoceptors are likely to be involved in this pressor response.

To demonstrate that the dose of pirenzepine used to block this central cholinergic pressor effect remained selective for M_1 cholinoceptors, we checked that this dose was insufficient to abolish two well-characterized cholinergic responses known to be induced by the stimulation of M_2 and M_3 muscarinic cholinoceptor subtypes: many higher doses of pirenzepine (40 to 50-fold higher than atropine) were indeed necessary to block the peripheral muscarinic M_2 receptor-induced bradycardia and the vasodilation induced by endothelial muscarinic M_3 receptors.

These findings confirm that the pressor response elicited by the intracisternal injection of acetylcholine is mediated through the activation of the muscarinic M_1 cholinoceptor subtype in anesthetized Beagle–Harrier dogs.

This result is different from previous findings in other experiments (Xiao and Brezenoff, 1988; Martin, 1992; Ally et al., 1995). This diversity of responses to central cholinergic blockade is probably related to several factors, including species specificity, drug selectivity, and the advancement of the pharmacology of the muscarinic cholinoceptors. In the conscious cat, Ally et al. (1995) clearly demonstrated that intracerebroventricular (i.c.v.) physostigmine (a cholinesterase inhibitor) elicited a dose-dependent pressor response which was blocked by methoctramine, a muscarinic M_2 cholinoceptor antagonist. Brezenoff et al. (1988) reported in the rat that i.c.v. injection of the muscarinic M_1 cholinoceptor antagonist pirenzepine failed to prevent the pressor response to oxotremorine. Moreover, the same authors reported that blockade of central muscarinic M_3 cholinoceptors (muscarinic M_2 cholinoceptors according to the nomenclature in 1988) with 4-diphenylacetoxy-*N*-methyl-piperidine methiodide (4-DAMP) inhibited the pressor response induced by i.v. physostigmine in urethane-anesthetized normotensive rats. Since 1992, 4-DAMP has been reclassified as muscarinic M_1/M_3 cholinoceptor antagonist (Martin, 1992; Eglen and Watson, 1996). It is likely that these authors would have rather presently concluded to a muscarinic M_1 and/or M_3 cholinoceptor participation in the physostigmine pressor response, as suggested by Martin (1992). Additionally, i.c.v. administration of 4-DAMP (25 μg) was shown to reduce blood pressure in spontaneously hypertensive rats (Brezenoff et al., 1988) Moreover, a participation of muscarinic M_1 cholinoceptor subtypes in the genesis of arterial hypertension in spontaneous hypertensive rats has also been evoked by binding studies and quantitative mRNAs analysis (Wei et al., 1995). The pressor response induced by central or peripheral administration of cholinergic agonists has been shown to involve caudal as well as more rostral brain areas (Brezenoff and Giuliano, 1982). In agreement with this last assumption, Hori et al. (1995) reported that intrahippocampal injection of neostigmine, in normotensive rats, induced a pressor response prevented by pirenzepine; other brain areas, however, can be involved.

In our experiments, although the response obtained after i.c. injection of muscarinic antagonists may represent the contribution of receptors located in different rostral brain areas, it is tempting to suggest that the cardiovascular effects of acetylcholine injected by intracisternal route are related to muscarinic mechanisms exerted at the level of the lower brainstem and/or the spinal cord. Indeed, these areas have been shown to contain several cholinergic neurons as well as muscarinic receptors involved in the blood pressure control (Buccafusco, 1996).

Our data further showed that high doses of methoctramine (20 $\mu g\ kg^{-1}$) induced an increase in systolic and diastolic blood pressure. This pressor response can be explained by a vasopressin release because we observed simultaneously a significant increase in its plasma levels

(+22 pg ml^{-1}). One can wonder, however, if this vasopressin plasma levels increase was sufficient to produce a vasoconstriction. In fact, Rossi and Schrier (1986) reported that 40 pg ml^{-1} was necessary to induce a pressor response in dogs. Another possibility could be that high doses of the muscarinic M_2 cholinoceptor antagonist, methoctramine, have been reported to block presynaptic inhibitory autoreceptors, thus blocking the inhibition on acetylcholine release in the synaptic cleft (Özkutlu et al., 1993).

In conclusion, this study demonstrates that in anesthetized dog, the central administration of acetylcholine evokes an increase in systolic and diastolic blood pressure and in noradrenaline and vasopressin plasma levels. The cardiovascular effects of acetylcholine, 30 μg kg^{-1} i.c., are mediated, in dogs, by the muscarinic M_1 cholinoceptor subtype. It would be interesting to study the effect of intracisternal injection of specific muscarinic M_1 cholinoceptor agonists like FKS-508 or SB 202026 which are presently proposed as potential antidementia agents in patients with Alzheimer's disease.

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