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Pressor and bradycardic effects of tacrine and other acetylcholinesterase inhibitors in the rat

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Abstract

The cardiovascular effects of three different acetylcholinesterase inhibitors: physostigmine, tacrine and rivastigmine injected by intravenous (i.v.) route were compared in freely moving Wistar rats. The three drugs significantly increased both systolic and diastolic blood pressure and decreased heart rate. Compared to physostigmine, a 20-fold higher dose of tacrine and a 40-fold higher dose of rivastigmine was necessary to induce a comparable pressor effect. Tacrine was chosen as a model to study the mechanisms underlying the cardiovascular effects of i.v. cholinesterase inhibitors. Atropine totally abolished while methylatropine did not affect tacrine pressor effects. Conversely, both drugs abolished tacrine-induced bradycardia. The α_1-adrenoceptor antagonist prazosin or the vasopressin V_1 receptor antagonist, [β-mercapto-β,β-cyclopenta-methylenepropionyl[1], *O*-Me-Tyr[2], Arg[8]] vasopressin partially but significantly reduced tacrine pressor effect and mostly abolished it when administered concomitantly. The tacrine pressor response was inhibited in a dose-dependent manner by the i.c.v. administration of the non-selective muscarinic receptor antagonist atropine ($ID_{50} = 1.45$ μg), the muscarinic M_1 receptor antagonist pirenzepine ($ID_{50} = 4.33$ μg), the muscarinic M_2 receptor antagonist methoctramine ($ID_{50} = 1.39$ μg) and the muscarinic M_3 receptor antagonist *para*-fluoro-hexahydro-sila-difenidol ($ID_{50} = 31.19$ μg). Central injection of such muscarinic receptor antagonists did not affect tacrine-induced bradycardia. Our results show that acetylcholinesterase inhibitors induce significant cardiovascular effects with a pressor response mediated mainly by the stimulation of central muscarinic M_2 receptors inducing a secondary increase in sympathetic outflow and vasopressin release. Conversely, acetylcholinesterase inhibitor-induced bradycardia appears to be mediated by peripheral muscarinic mechanisms.

Keywords: Acetycholinesterase inhibitor; Blood pressure; Vasopressin

1. Introduction

Several cholinomimetics are developed or already marketed for the symptomatic treatment of the most common form of dementia, namely Alzheimer's disease, because it is believed that a central acetylcholine deficit explains part of the characteristic memory deficits observed in patients suffering from this disease (Wagstaff and McTavish, 1994). For example tacrine (tetrahydroaminoacridine) and rivastigmine (SDZ ENA713), two reversible inhibitors of acetylcholinesterase, have been reported to improve cognitive function and behavioural deficits in patients suffering from Alzheimer's disease (Summers et al., 1986; Davis et al., 1992) and in animal models of cognitive deficits (Ohara et al., 1997).

Central cholinergic systems do not only control memory processes. They are also involved in other important physiological functions. Several experimental arguments suggest, for example, that acetylcholine participates in the central control of blood pressure (Brezenoff and Giuliano,

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1982; Buccafusco, 1996). This central cholinergic pressor effect is assumed to be mediated, at the periphery, by an increase in sympathetic tone (Krstic and Djurkovic, 1978; Buccafusco and Brezenoff, 1979) while the role of a vasopressin release remains discussed (Kawashima et al., 1986; Rascol et al., 1990; Savci et al., 1998). The subtype of muscarinic cholinoceptors which mediate this central cholinergic response remains largely debated, some authors having suggested the involvement of M_1 (Hori et al., 1995), M_2 (Özkutlu et al., 1993; Ally et al., 1995) or M_3 (Martin, 1992) subtypes. These discrepancies might be due to differences in species or injection sites.

Such cardiovascular cholinergic effects are important to study because they suggest that there is a risk for potent acetylcholinesterase inhibitors, like those developed for the treatment of Alzheimer's disease, to induce significant cardiovascular side effects. Indeed, pharmacovigilance studies have shown that bradycardia is an adverse drug reaction already reported with tacrine in humans (unpublished data from the French Pharmacovigilance Database). Recently, a severe increase in blood pressure induced by a tacrine treatment has also been reported in a patient suffering from Alzheimer's disease (Allain et al., 1996). A comparable observation has also been reported with physostigmine (Cain, 1986).

The aims of the present study are (1) to investigate, in freely moving rats, the cardiovascular changes induced by an intravenous (i.v.) injection of three different acetylcholinesterase inhibitors: tacrine (because it is the first acetylcholinesterase inhibitor marketed for the treatment of Alzheimer's disease), physostigmine (because it is the reference acetylcholinesterase inhibitor compound in the literature) and rivastigmine (because it is a new acetylcholinesterase inhibitor developed for the treatment of Alzheimer's disease), and (2) to identify the central and peripheral mechanisms involved in this response, looking for the subtype(s) of cholinoceptor(s) implicated in such cardiovascular responses and the respective role of the sympathetic nervous system and/or vasopressin release in mediating these effects.

2. Materials and methods

2.1. Animals

Experiments were performed on Wistar freely moving male rats weighing 250 to 350 g. These animals were obtained from IFFA CREDO (L'Arbresle, France) and were maintained at 20–24°C with a 12 h light–dark cycle (light on 0800–2000 h) at least 1 week before the experiment. Food pellets and tap water were available ad libitum. All animals procedures were conducted in strict compliance with approved French Agriculture Department for Animal Use for Research and Education groups.

2.2. Surgical preparation

2.2.1. Peripheral injections

The rats were initially anesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.) to insert venous and arterial catheters allowing subsequent recording of cardiovascular parameters and drug administration. For this purpose, animals were placed in a blanket, connected to a rectal probe, to avoid heat loss and body temperature was maintained at 38°C (Harvard Apparatus, England). The right external jugular vein and the left carotid artery were cannulated with a polyethylene catheter (PE-50). The catheters were then tunneled subcutaneously to the nape of the neck, where they were exteriorized. After this, each rat was allowed at least 2 days to recover. Arterial and venous catheters were kept patent by daily administration of 0.1 ml of heparin (100 UI ml^{-1}) in 0.9% saline.

2.2.2. Intracerebroventricular injections

The rats were anesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.) then the right external jugular vein and the left carotid artery were cannulated as previously described.

Rats were then placed in a stereotaxic frame (Unimecanique, Paris, France) for central drug administration and a cannula was implanted, according to the atlas of Paxinos and Watson (1982), in the right lateral cerebroventricle using the following coordinates relative to bregma: posterior 1.0 mm, lateral 1.5 mm, ventral 4.0 mm from the surface of the skull. The cannula was connected to a 25 μl Hamilton syringe with polyethylene (PE-50) tubing. The injection cannula was filled by backfilling with 10 μl of injectate. The syringe itself was filled with distilled water and an air bubble was left between water and drug solution. Methylene blue was injected i.c.v. after each experiment for verification of the cannula placement and only proper intracerebroventricular (i.c.v.) placements were included in the study.

2.2.3. Cardiovascular parameter-recording

The day of the experiment, arterial and venous catheters were connected to a pressure transducer and to a syringe, respectively. Then, the rat was left alone in a rodent sampling cage so that blood pressure could stabilize. Blood pressure was continuously recorded via a pressure transducer (Abbott, Transpac IV, Ireland) and amplifier (Bionic Instruments, Qazap 94104, France) coupled to a MacLab hardware unit (ADInstruments, MacLab/4S, Australia) connected to a microcomputer (PowerMacintosh 6200, Apple, USA). Heart rate was triggered by the blood pressure signal and expressed in beats per min (bpm). The sampling rate was 10 Hz and data were collected on a 20 s period. Respiratory rate was also recorded, by observation of the chest movements, and expressed in breaths per min.

Table 1
Effects of various doses of acetylcholinesterase inhibitors (physostigmine, tacrine and rivastigmine) on blood pressure and heart rate, in freely moving Wistar rats, following i.v. administration

	Maximal increase in systolic blood pressure (mm Hg)	Maximal increase in diastolic blood pressure (mm Hg)	Maximal decrease in heart rate (bpm)
Physostigmine 50 μg kg^{-1}	+31 ± 5	+23 ± 8	−22 ± 11
Tacrine 0.5 mg kg^{-1}	+5 ± 2	+1 ± 2	−7 ± 5
Tacrine 1 mg kg^{-1}	+31 ± 3	+19 ± 2	−29 ± 5
Tacrine 2 mg kg^{-1}	+46 ± 4	+25 ± 6	−47 ± 9
Rivastigmine 1 mg kg^{-1}	+14 ± 3	+10 ± 2	−26 ± 12
Rivastigmine 2 mg kg^{-1}	+34 ± 5	+22 ± 6	−28 ± 10

Values are expressed as means ± S.E.M.

2.3. Cardiovascular effects of the three acetylcholinesterase inhibitors

In the first part of this study, we assessed the cardiovascular effects of a bolus i.v. injection of three different acetylcholinesterase inhibitors: physostigmine (50 μg kg^{-1}), tacrine (1 mg kg^{-1}) and rivastigmine (2 mg kg^{-1}) in freely moving rats. These doses were assessed because 50 μg kg^{-1} of physostigmine is usually reported to induce significant cardiovascular effects in the literature (Brezenoff and Giuliano, 1982; Oktay et al., 1984; Tellioglu et al., 1996). Then, we performed dose–response experiments to define the dose of tacrine and rivastigmine which induces cardiovascular changes of the same range than 50 μg kg^{-1} of physostigmine (Table 1). The acetylcholinesterase inhibitor injection was preceded, 5 min before, by an i.v. saline injection (0.3 ml) in order to test non-specific cardiovascular effects. Blood pressure, heart rate and respiratory rate were monitored during 60 min after administration.

2.4. Mechanisms of the cardiovascular effects of tacrine

In order to determine the central and/or peripheral mechanisms underlying the effects of acetylcholinesterase inhibitors, we tested the pressor response to i.v. tacrine administration (1 mg kg^{-1}) in seven different conditions.

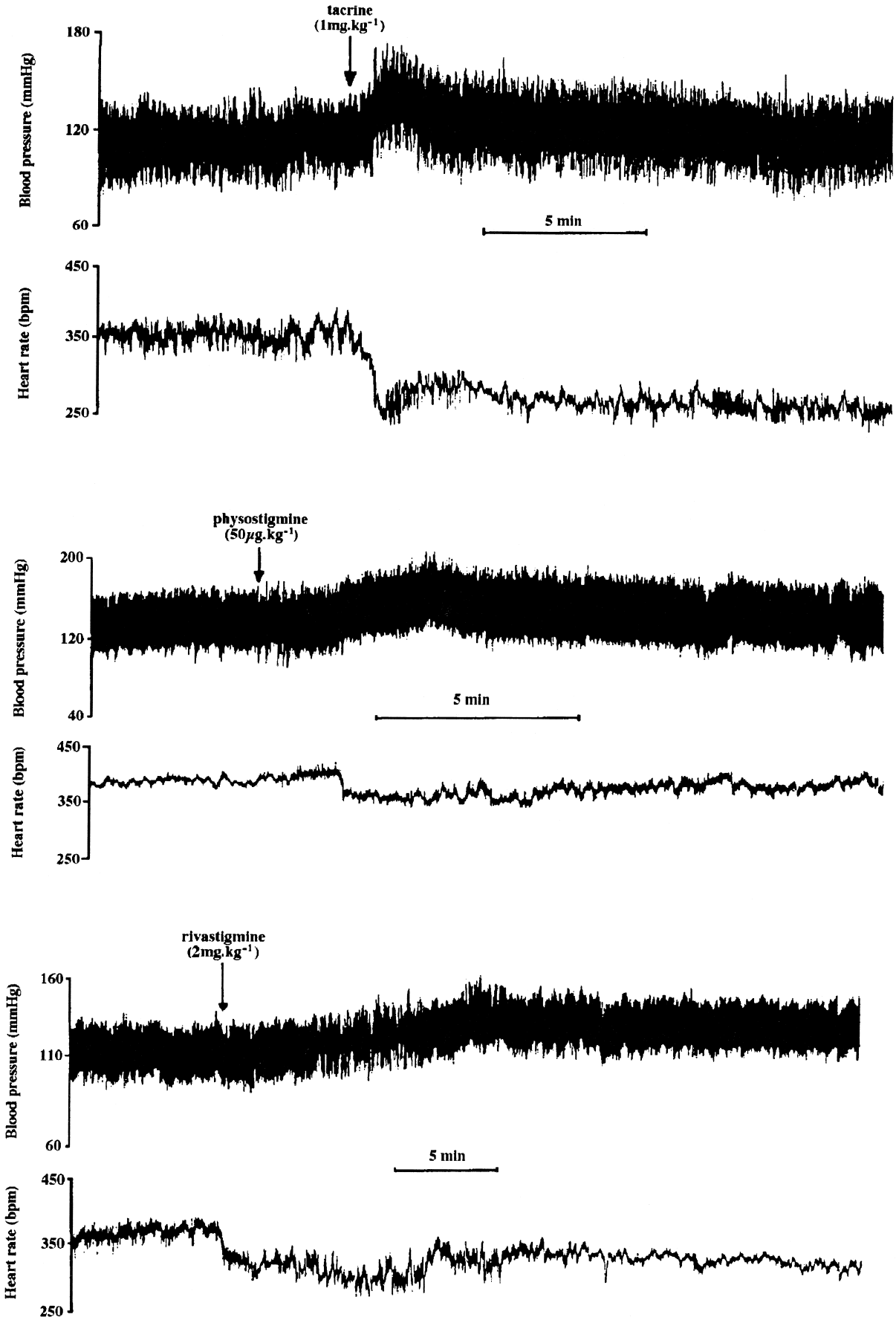
In the 'saline' group, the rats received two i.v. injections of a similar volume of saline, in order to evaluate the effects of repeated systemic injections. The 'methylatropine' and 'atropine' groups were designed to assess the involvement of peripheral and central muscarinic receptors in the tacrine's effects. The 'prazosin', 'vasopressin V_1 receptor antagonist' and 'prazosin + vasopressin V_1 receptor antagonist' groups were designed to assess the respective involvement of catecholamines and vasopressin release in the pressor response to tacrine. In the 'chlorisondamine' group, we assessed the effects of a ganglionic blockade on the tacrine-induced cardiovascular response.

Each treatment group was assessed by at least six separate experiments each in different animals. In the 'methylatropine', 'atropine', 'prazosin', 'vasopressin V_1 receptor antagonist' and 'chlorisondamine' groups, each antagonist was injected by i.v. route 5 min before the systemic administration of tacrine. In the 'prazosin + vasopressin V_1 receptor antagonist' group, prazosin was the first drug injected, followed by the vasopressin receptor antagonist and, five min later, tacrine. All cardiovascular and respiratory parameters were measured 1 min before and 3 min after the i.v. injection of the antagonists

Table 2
Baseline cardiovascular parameters in the different treatment groups of Wistar rats before any pretreatment

	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Heart rate (bpm)
Saline ($n = 8$)	142 ± 4	109 ± 2	374 ± 14
Tacrine ($n = 7$)	153 ± 7	122 ± 6	346 ± 11
Physostigmine ($n = 6$)	147 ± 3	112 ± 3	357 ± 6
Rivastigmine ($n = 6$)	143 ± 7	112 ± 6	366 ± 11
Methylatropine ($n = 8$)	146 ± 6	115 ± 6	395 ± 14
Atropine ($n = 8$)	148 ± 4	106 ± 6	350 ± 7
Prazosin ($n = 7$)	150 ± 3	110 ± 3	366 ± 7
Vasopressin V_1 receptor antagonist ($n = 6$)	149 ± 5	105 ± 7	354 ± 15
Prazosin + vasopressin V_1 receptor antagonist ($n = 6$)	146 ± 2	108 ± 3	365 ± 15
Chlorisondamine ($n = 6$)	149 ± 4	106 ± 3	343 ± 6

Values are expressed as means ± S.E.M.


tacrine
(1mg.kg⁻¹)
Blood pressure (mmHg)
180
120
60
5 min
Heart rate (bpm)
450
350
250
physostigmine
(50μg.kg⁻¹)
Blood pressure (mmHg)
200
120
40
5 min
Heart rate (bpm)
450
350
250
rivastigmine
(2mg.kg⁻¹)
Blood pressure (mmHg)
160
110
60
5 min
Heart rate (bpm)
450
350
250

to assess if they induced by themselves any effects. All i.v. injections were administered in a volume of 0.25–0.35 ml.

2.5. Subtypes of muscarinic cholinoceptors involved

In order to determine the subtype(s) of central muscarinic cholinoceptor(s) involved in the tacrine's pressor response (1 mg kg^{-1}), we administered saline and various muscarinic antagonists by i.c.v. route, 10 min prior to the tacrine i.v. injection. Three doses of each antagonist (atropine: 1, 2.5 and 5 μg–pirenzepine: 2.5, 5 and 10 μg–methoctramine: 1, 2.5 and 5 μg–*para*-fluoro-hexahydro-sila-difenidol (*p*-F-HHSiD): 10, 25 and 50 μg) were tested in at least six different animals.

2.6. Drugs

The following drugs were used: sodium pentobarbitone (Pentobarbital sodique®: Sanofi), physostigmine salicylate salt (a centrally acting acetylcholinesterase inhibitor), atropine sulfate (a non-selective muscarinic receptor antagonist which crosses blood–brain barrier), methylatropine sulfate (a non-selective peripheral muscarinic receptor antagonist), prazosin hydrochloride (an α_1-adrenoceptor antagonist), [β-mercapto-β,β-cyclopenta-methylenepropionyl[1], *O*-Me-Tyr[2], Arg[8]] vasopressin (a vasopressin V_1 receptor antagonist), 9-amino-1,2,3,4-tetrahydroacridine hydrochloride (or tacrine) (Sigma–Aldrich, St. Quentin Fallavier, France). (*S*)-*N*-ethyl-*N*-methylcarbamic acid 3-[1-(dimethylamino)ethyl]phenyl ester (rivastigmine) was a gift from Novartis Pharma. Pirenzepine (a muscarinic M_1 receptor antagonist), methoctramine (a muscarinic M_2 receptor antagonist), *p*-F-HHSiD (*para*-fluoro-hexahydro-sila-difenidol, a muscarinic M_3 receptor antagonist) and 2-hydroxypropyl-β-cyclodextrin (Research Biochemicals, Natick, USA). Chlorisondamine (a ganglionic blocker) was a gift from Ciba-Geigy. The doses of atropine (0.4 mg kg^{-1}), methylatropine (0.2 mg kg^{-1}), vasopressin V_1 receptor antagonist (20 μg kg^{-1}) and prazosin (1 mg kg^{-1}) were chosen according to their ability to block in our experimental model the peripheral effects of acetylcholine (2.5 μg kg^{-1}), vasopressin (100 ng kg^{-1}) and phenylephrine (5 μg kg^{-1}), respectively (data not shown). Such doses of acetylcholine, vasopressin and phenylephrine induced blood pressure changes in the range of the vascular effects induced by tacrine (~30 mm Hg). Prazosin and *p*-F-HHSiD were dissolved in methanol and 2-hydroxypropyl-β-cyclodextrin, respectively (in each case, the vehicle was tested to make sure that it did not induce, by itself, any effect on blood pressure and heart rate). All other drugs were dissolved in physiological saline. All given doses of drugs refer to the free base.

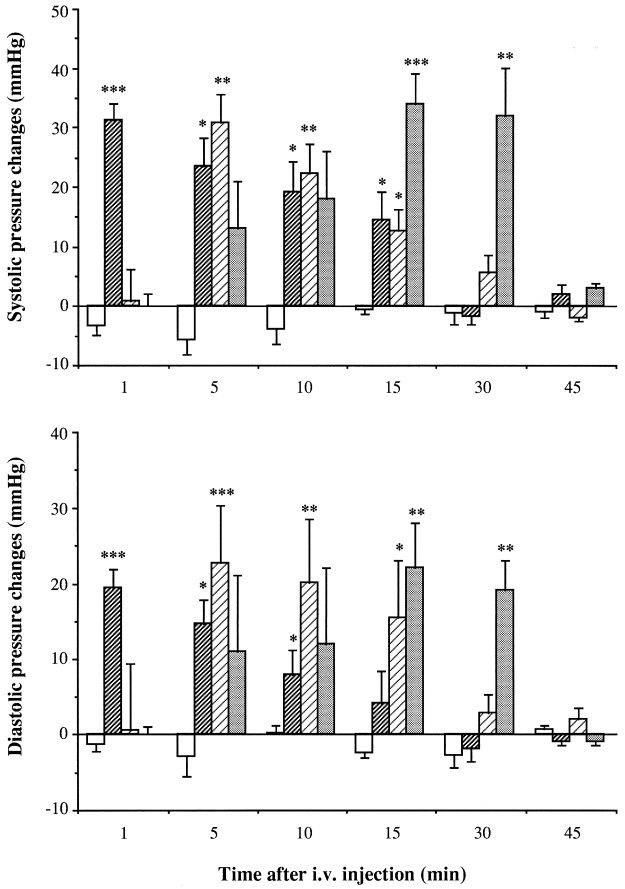


Fig. 2. Mean changes in systolic and diastolic blood pressure elicited by intravenous injection of saline (0.3 ml, open columns), tacrine (1 mg kg^{-1}, closely hatched columns), physostigmine (50 μg kg^{-1}, widely hatched columns) and rivastigmine (2 mg kg^{-1}, shaded columns) in freely moving Wistar rats. Values are expressed as variation of the mean ± S.E.M. Statistical significance vs. the saline group: $* P < 0.05$, $** P < 0.01$ and $*** P < 0.001$.

2.7. Statistical methods and data analysis

The experimental data were obtained from six to eight animals for each treatment group, each experiment being performed in a different animal.

A one-way analysis of variance (ANOVA) was performed to compare the baseline means of the different parameters (blood pressure and heart rate) in each group in order to assess if there was any significant intergroup difference on baseline 5 min before any injection.

A one-way ANOVA for repeated measures was used to compare the different parameters at the different times in each group in order to assess specific (drugs) or non-specific (saline) effects within these groups.

The paired-sample Student's *t*-test was used to compare the means of the different parameters 1 min before and 2

Fig. 1. Typical example of the cardiovascular changes in the blood pressure and heart rate after intravenous injection of tacrine (1 mg kg^{-1}), physostigmine (50 μg kg^{-1}) and rivastigmine (2 mg kg^{-1}) in freely moving Wistar rats.

min after i.v. antagonists in order to assess if these drugs induced any effects by themselves.

According to the homogeneity of variances (Bartlett's test), a two-way multivariate ANOVA was used to compare the mean's variations (Δ) of the different parameters in the different groups at the different times (1, 5, 10, 15, 30 and 45 min) in order to assess if there was any significant different effect in these groups. The Scheffe's test and the Dunnett's test were used as post hoc tests for intergroup and intragroup comparisons, respectively.

Dose–response curves were fitted by non-linear regression to a sigmoïda and ID_{50} calculated using the program Prism (Graph Pad 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

Baseline cardiovascular and respiratory parameters were not significantly different before i.v. injections in any group (Table 2). Saline induced no significant change in any parameter after i.v. or i.c.v. injection at any time. Physostigmine, tacrine and rivastigmine i.v. injections induced moderate micturition, defecation and licking in most animals.

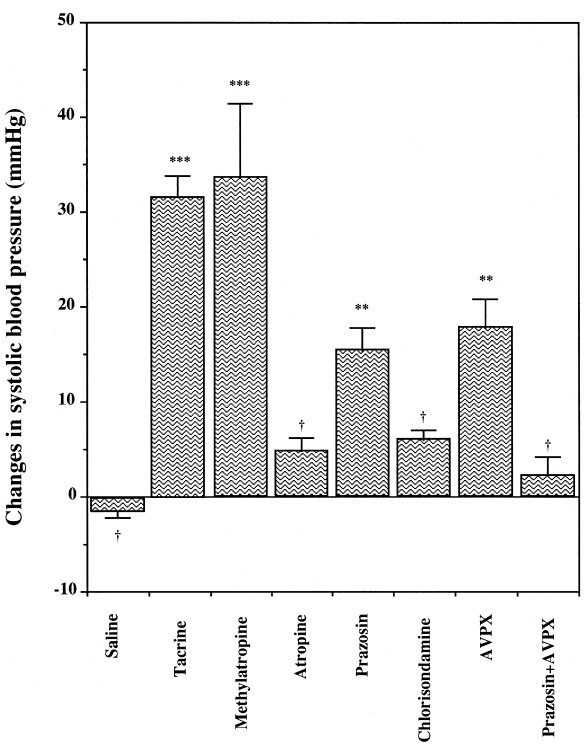


Fig. 3. Changes in systolic blood pressure elicited 1 min after intravenous injection of tacrine (1 mg kg^{-1}, $n = 7$) or saline ($n = 8$) in Wistar freely moving rats. In the other groups, the animals were pretreated 5 min before tacrine administration, by methylatropine (0.2 mg kg^{-1}, $n = 8$), atropine (0.4 mg kg^{-1}, $n = 8$), prazosin (1 mg kg^{-1}, $n = 7$), chlorisondamine (2.5 mg kg^{-1}, $n = 6$), vasopressin V_1 receptor antagonist (20 μg kg^{-1}, $n = 6$) and prazosin + vasopressin V_1 receptor antagonist ($n = 6$). A VPX was used as abbreviation for vasopressin V_1 receptor antagonist. Values are expressed as mean variations ± S.E.M. Statistical significance vs. the saline group: $**P < 0.01$ and $***P < 0.001$. Statistical significance vs. the tacrine group: $†P < 0.05$.

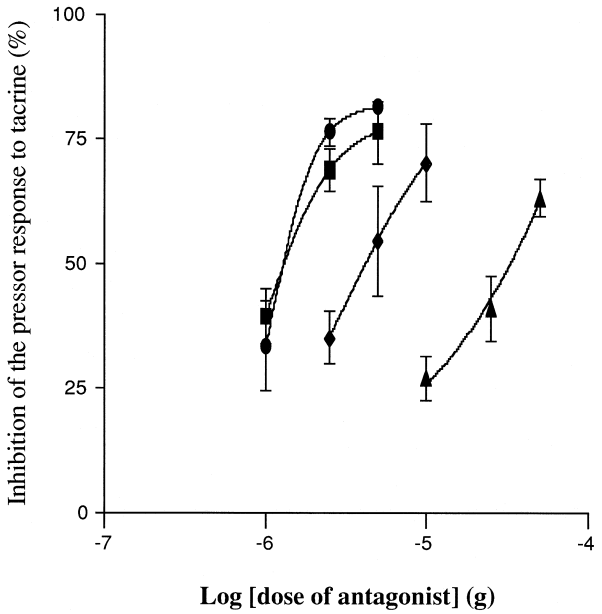


Fig. 4. The pressor response of i.v. tacrine (1 mg kg^{-1}) was inhibited by appropriate amounts of the no selective muscarinic antagonist atropine (1–5 μg, ●) or the selective muscarinic antagonists pirenzepine (M_1 antogonist, 2.5–10 μg, ♦), methoctramine (M_2 antagonist, 1–5 μg, ■) and *p*-F-HHSiD (M_3 antagonist, 10–50 μg, ▲). All points consist of five to six animals. Results are expressed as means ± S.E.M.

3.1. Cardiovascular responses induced by the three acetylcholinesterase inhibitors in freely moving rats

Fig. 1 shows a typical example of the effects of an i.v. injection of each acetylcholinesterase inhibitor.

Physostigmine (50 μg kg^{-1}) induced a progressive and significant increase in systolic and diastolic blood pressure, which reached its maximal value after 5 min ($+31 \pm 4$ mm Hg, ANOVA $P < 0.01$ and $+23 \pm 8$ mm Hg, ANOVA $P < 0.001$, respectively) and lasted over 15 min before returning to basal values within 30 min (Fig. 2).

Tacrine, at the dose of 1 mg kg^{-1}, induced a significant increase in systolic and diastolic blood pressure of the same amplitude ($+31 \pm 3$ and $+19 \pm 2$ mm Hg, respectively, ANOVA $P < 0.001$). This increase in blood pressure occurred within 1 min and remained significant during 15 min.

Rivastigmine at the dose of 2 mg kg^{-1} induced a similar increase in systolic and diastolic blood pressure ($+34 \pm 5$ mm Hg, ANOVA $P < 0.001$ and $+22 \pm 6$ mm Hg, ANOVA $P < 0.01$, respectively), reaching its maximal value after only 15 min and remaining significant up to 30 min.

The magnitude of the pressor responses were similar for the three drugs (around +30 mm Hg). Moreover, there were no significant differences for the area under the curve between the three drugs (data not shown).

For each acetylcholinesterase inhibitor, the blood pressure increase was associated with a significant bradycardia appearing in the five first min and lasting 10 min, with maximal changes of -22 ± 11, -29 ± 5 and -28 ± 10 bpm, respectively (ANOVA $P < 0.05$).

3.2. Effects of pretreatment with cholinergic, noradrenergic and vasopressinergic antagonists on baseline parameters

The i.v. injection of methylatropine, atropine, vasopressin V_1 receptor antagonist and the i.c.v. injection of atropine, pirenzepine and *p*-F-HHSiD induced no significant changes in any measured parameter (data not shown). Conversely, i.v. prazosin induced by itself a significant decrease in mean blood pressure (-41 ± 3 mm Hg, $P < 0.01$) and a significant increase in heart rate ($+30 \pm 5$ bpm, $P < 0.05$). I.v. chlorisondamine induced a significant decrease in both mean blood pressure and heart rate (-51 ± 7 mm Hg and -40 ± 3 bpm, respectively, $P < 0.05$) while i.c.v. methoctramine induced a significant increase in mean blood pressure ($+17 \pm 3$ mm Hg, $P < 0.05$) without affecting heart rate (values measured after 2 min).

3.3. Peripheral mechanisms underlying the cardiovascular changes induced by tacrine

The effects of the α_1-adrenergic and vasopressinergic V_1 receptor antagonists on tacrine pressor response were measured 1 min after tacrine injection (peak of tacrine pressor response). The two-way ANOVA showed that there was a significant time by group interaction ($P < 0.001$) (Fig. 3).

The tacrine-induced increase in systolic and diastolic blood pressure was not different in the 'tacrine' ($+31 \pm 3$ and $+19 \pm 2$ mm Hg, respectively) and 'methylatropine' ($+34 \pm 8$ and $+19 \pm 4$ mm Hg, respectively) groups, but was significantly reduced in the 'atropine' one ($+5 \pm 1$ and $+5 \pm 2$ mm Hg, respectively, $P < 0.05$).

Tacrine's pressor effects were also significantly reduced in the 'prazosin' ($+15 \pm 2$ and $+14 \pm 1$ mm Hg, respectively, $P < 0.05$) and 'chlorisondamine' ($+6 \pm 1$ and $+4 \pm 2$ mm Hg, respectively, $P < 0.05$) groups when compared to the 'tacrine' one.

Similarly, tacrine pressor response was significantly reduced in the 'vasopressin V_1 receptor antagonist' ($+18 \pm 3$ and $+9 \pm 2$ mm Hg, respectively, $P < 0.05$) group.

Moreover, in the 'prazosin + vasopressin V_1 receptor antagonist' group, tacrine's pressor response was nearly

Table 3
Rats were pretreated with the non-selective muscarinic receptor antagonist, atropine, the selective M_1 muscarinic receptor antagonist, pirenzepine, the selective M_2 muscarinic receptor antagonist, methoctramine and the selective M_3 muscarinic receptor antagonist, *p*-F-HHSiD

Pretreatment	Saline	Atropine			Pirenzepine			Methoctramine			*p*-F-HHSiD		
Dose	10 µl	1 µg	2.5 µg	5 µg	2.5 µg	5 µg	10 µg	1 µg	2.5 µg	5 µg	10 µg	25 µg	50 µg
Systolic blood pressure (mm Hg)	$+30 \pm 3$	$+20 \pm 3$	$+7 \pm 1^b$	$+6 \pm 1^b$	$+16 \pm 2^a$	$+13 \pm 3^b$	$+9 \pm 2^b$	$+18 \pm 4^b$	$+9 \pm 1^b$	$+7 \pm 2^b$	$+19 \pm 2$	$+17 \pm 2$	$+11 \pm 1^b$
Diastolic blood pressure (mm Hg)	$+9 \pm 2$	$+11 \pm 4$	$+4 \pm 1$	$+2 \pm 1$	$+6 \pm 2$	$+6 \pm 2$	$+7 \pm 2$	$+10 \pm 1$	$+2 \pm 2$	$+5 \pm 2$	$+6 \pm 2$	$+7 \pm 1$	$+7 \pm 2$
Heart rate (bpm)	-31 ± 6	-41 ± 6	-31 ± 10	-36 ± 9	-55 ± 12	-44 ± 11	-40 ± 11	-57 ± 11	-50 ± 18	-27 ± 5	-71 ± 25	-61 ± 15	-46 ± 8
log ID_{50} (g)	–		-5.84 ± 0.05			-5.36 ± 0.08			-5.86 ± 0.07			-4.51 ± 0.06	
ID_{50} (µg)	–		1.45 ± 0.16			4.33 ± 0.7			1.39 ± 0.22			31.19 ± 4.28	
Hill slope	–		1.60 ± 0.34			1.06 ± 0.36			1.06 ± 0.23			0.96 ± 0.21	

Differences between groups were tested for statistical significance by Scheffe's post hoc test ($P < 0.05$ was considered significant). [a] $P < 0.05$; [b] $P < 0.001$. Each group consist of six animals. Data were calculated by ANOVA and presented as means ± S.E.M.

abolished ($+2 \pm 2$ and $+9 \pm 5$ mm Hg, respectively, $P < 0.05$) when compared to the tacrine group.

3.4. Central muscarinic receptors subtypes involved in the pressor response to tacrine

The pretreatment of the rats by an i.c.v. injection of 1 to 5 μg of atropine resulted in a dose-dependent inhibition of the peak tacrine (1 mg kg^{-1}) effects on blood pressure ($P < 0.05$) (Fig. 4). Dose–response curves analysis showed that atropine had an ID_{50} equal to 1.45 μg (Table 3).

Pretreatment with pirenzepine (2.5–10 μg i.c.v.) inhibited in a dose-dependent way the blood pressure response. The ID_{50} calculated from the dose–response curves was equal to 4.33 μg ($P < 0.05$, Table 3).

Methoctramine pretreatment (1–5 μg i.c.v.) inhibited in a dose-dependent way the increase in blood pressure evoked by 1 mg kg^{-1} i.v. of tacrine with an ID_{50} equal to 1.39 μg ($P < 0.05$, Table 3).

The tacrine-induced pressor response was dose-dependently inhibited by *p*-F-HHSiD (10–50 μg i.c.v.), the ID_{50} being equal to 31.19 μg ($P < 0.05$, Table 3).

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

In each treatment group and for each dose used, bradycardia associated to the pressor response to tacrine was not altered by i.c.v. pretreatment with the muscarinic receptor antagonists (Table 3).

4. Discussion

The present study demonstrates that three different acetylcholinesterase inhibitors, namely physostigmine, tacrine and rivastigmine induced significant changes in blood pressure and heart rate in freely moving Wistar rats when injected by i.v. route. Tacrine being chosen as an example of the acetylcholinesterase inhibitor pharmacological family, our results show that the pressor response to this drug was mediated by the stimulation of central muscarinic M_2 receptors, inducing a peripheral increase in both sympathetic outflow and vasopressin release, while its bradycardic effect was mediated by peripheral muscarinic mechanisms.

4.1. Description and comparison of the cardiovascular effects induced by the three different acetylcholinesterase inhibitors

4.1.1. Pressor effects

At the appropriate dose of 50 μg kg^{-1} of physostigmine, 1 mg kg^{-1} of tacrine and 2 mg kg^{-1} of rivastigmine, the three drugs induced a maximal increase in blood pressure response of about +30 mm Hg. The duration of these blood pressure responses, also turned out to be similar with the three drugs, lasting 15 min on average. However, the onset of the response was quite different, from one drug to the other, the tacrine response occurring within a quite short delay (maximal effect after 1 min) while that of physostigmine and rivastigmine effects occurred later on: maximal effect after 5 min and 15 min, respectively (Fig. 2). We have no definite explanation for these different delays to reach the peak effects with the three drugs which might involve differences in crossing blood–brain barrier. Nevertheless, these pressor effects of the three acetylcholinesterase inhibitors are in good agreement with what has already been published with other comparable drugs or cholinomimetic agents (Brezenoff and Giuliano, 1982; Buccafusco, 1996).

Rivastigmine has recently been claimed to have a larger therapeutic window than tacrine when treating patients with Alzheimer's disease (Weinstock, 1997). Our data might support this assumption, although it is difficult to extrapolate from a single dose study like the present one. However, one can note that there is an inverse relative potency of tacrine and rivastigmine when considering their cognitive and pressor effects. The present data show that the acute dose of rivastigmine must be twice larger than that of tacrine to induce the same increase in blood pressure, while previously published data show that the daily dose of rivastigmine is more than 10-fold smaller than that of tacrine to induce a clinical improvement on cognitive functions in patients with Alzheimer's disease. Indeed, the usual recommended daily dose of tacrine is 80 to 160 mg/day (Hollister and Gruber, 1996) while it is only 6 to 12 mg/day for rivastigmine (Sramek et al., 1996). Both drugs have a comparable oral bioavailability of about 25% (Parnetti, 1995; Novartis in-house data). Therefore, differences in peripheral pharmacokinetic mechanisms are unlikely to explain the different relative potencies of both drugs on both functions. A different ability of the two drugs to cross the blood–brain barrier cannot either be advocated because both pressor and cognitive cholinergic mechanisms are located within the blood–brain barrier. Therefore, it seems reasonable to suggest that rivastigmine might be more effective on cholinergic cognitive central mechanisms than on pressor ones. Indeed, it has been suggested that rivastigmine has a more selective action on acetylcholinesterase in the cortex and hippocampus (where cognitive mechanisms may be located) than in the pons-medulla (Weinstock et al., 1986, 1994; Weinstock, 1997), a brain area where the origin of the cholinergic pressor response is generally located (Brezenoff and Giuliano, 1982). From a molecular point of view, one can speculate that the smaller pressor potency of rivastigmine could be related to its selectivity for the G1 molecular form of brain acetylcholinesterase, in contrast to physostigmine and tacrine, which block both G1 and G4 forms with equal efficiency (Weinstock et al., 1994). However, we are not aware of any clearly demonstrated topographic differences in the central distribution of G1 and G4 acetylcholineste-

rase forms and no clear link between any specific G form and different physiological functions have been published up to now.

4.1.2. Effects on heart rate

The systemic injection of the three acetylcholinesterase inhibitors induced a very similar profile of heart rate changes from one drug to the other. The three drugs reduced heart rate with a similar range (−25 bpm on average), this effect being maximal within the five first min following the drug injection and lasting less than 10 min. This homogeneity in the heart rate response temporal profile to the three acetylcholinesterase inhibitors, is in marked contrast with the diversity of the temporal profile of the pressor response induced by the same three drugs. This observation suggests that the mechanisms underlying the pressor and bradycardic responses induced by acetylcholinesterase inhibitors might be independent.

4.2. Mechanisms of the acetylcholinesterase inhibitor-induced cardiovascular changes

4.2.1. Pressor effect

According to the similar changes in blood pressure induced by the three acetylcholinesterase inhibitors and in order to facilitate the experiments, tacrine was chosen as a model to study the mechanisms governing the cardiovascular effects of i.v. acetylcholinesterase inhibitors. The pressor response to i.v. tacrine was clearly mediated by central muscarinic cholinoceptors: it was antagonized by atropine, but not by methylatropine, a drug which does not cross the blood–brain barrier. The blockade of this response by the ganglionic blocker chlorisondamine, can also be seen as another evidence to involve central mechanisms. This finding is consistent with the well known central location of the cholinergic systems controlling blood pressure regulation (Brezenoff and Giuliano, 1982). This pressor effect of acetylcholinesterase inhibitors may involve various brain areas containing cholinergic neurons and cardiovascular pathways: the pons–medulla (Caputi et al., 1980; Lee et al., 1991), the hypothalamus (Buccafusco and Brezenoff, 1979; Xiao and Brezenoff, 1988) or maybe more rostral brain structures (Hori et al., 1995).

The muscarinic M_2 receptor antagonist, methoctramine, was as effective as atropine to prevent the increase in blood pressure induced by i.v. tacrine. It was 30-fold more potent than the muscarinic M_3 receptor antagonist, *p*-F-HHSiD, but only 3-fold more potent than the muscarinic M_1 receptor antagonist, pirenzepine. These findings suggest that central muscarinic M_2 cholinoceptors are mainly involved in the pressor response to i.v. tacrine. This study, to our best knowledge, is the first one to test the effects of different selective antagonists for the three muscarinic receptor subtypes on blood pressure response in the same experiment. Our data are in agreement with previously published results involving central muscarinic M_2 cholinoceptors in the cardiovascular effects of cholinomimetic drugs in the rat (Sundaram et al., 1988, 1989; Özkutlu et al., 1993) or the cat (Ally et al., 1993, 1995). Our results suggest that M_3 cholinoceptors are not involved in this response, in the rat, although conflicting opinions can be found in the literature (Xiao and Brezenoff, 1988; Brezenoff et al., 1988; Martin, 1992; Flores et al., 1996). Such differences may be due to the lack of specificity of some previously used 'selective' antagonists like 4-DAMP (4-Diphenylacetoxy-*N*-methylpiperidine) or to differences in central injection sites. The intermediate ID_{50} value obtained, in our study, with the muscarinic M_1 receptor antagonist pirenzepine cannot eliminate a weak involvement of muscarinic M_1 cholinoceptors in the pressor response to i.v. tacrine. Moreover, Hori et al. (1995) showed that the pressor response induced by the intrahippocampal administration of neostigmine in anesthetized rats was blocked by pretreatment with the muscarinic M_1 receptor antagonist, pirenzepine. Recently, a study conducted in the dog in our laboratory, showed that the pressor response to i.c. acetylcholine was also blocked by low doses of pirenzepine (Pelat et al., 1997).

The present data also demonstrate, as recently published by Savci et al. (1998), that the blockade of the peripheral α_1-adrenoceptors by prazosin resulted in a significant reduction of the tacrine-induced increase in blood pressure. Like these authors, we observed that the tacrine-induced pressor response was inhibited following the vasopressin V_1 receptor antagonist pretreatment, demonstrating that vasopressin release also participates in the pressor effect of tacrine. This result confirms previous studies of our laboratory performed in the dog (Rascol et al., 1990; Brefel et al., 1995) showing that plasma levels of noradrenaline, adrenaline and vasopressin are increased following central acetylcholine administration and supports the controversial role of vasopressin in mediating this pressor response (Buccafusco and Brezenoff, 1979; Brezenoff and Giuliano, 1982; Kawashima et al., 1986). This kind of interactions between vasopressinergic and cholinergic systems are well known, indeed, the release of vasopressin is under control by central cholinergic synapses (Kühn, 1974), however, the receptors (nicotinic or muscarinic) implicated in this release in the supraoptic and paraventricular nuclei are not clearly identified (Bisset and Chowdrey, 1984). Prazosin could also induce a rise in vasopressin plasma levels, when blood pressure is lowered, reducing the ability of the acetylcholinesterase inhibitor to rise blood pressure. Although a similar phenomenon was observed in dogs after administration of sodium nitroprusside (Lazartigues et al., 1998) and in rats made hypotensive by haemorrhage (Ulus et al., 1995), this possibility was not tested in the current experiments. In addition to Savci et al. (1998), our results also show that the combined pretreatment with an α_1-adrenoceptor antagonist and a vasopressin V_1 receptor antagonist nearly completely abolished the pressor response to tacrine while each drug individually only re-

duced the response by 50% on average. This observation suggests that noradrenergic and vasopressinergic systems are the two main efferent systems mediating the central pressor cholinergic response.

4.2.2. Bradycardia

The tacrine-induced bradycardia was clearly abolished by the peripheral administration of methylatropine and atropine, but was not influenced by the central administration of atropine or either antagonists. These data demonstrate that peripheral muscarinic mechanisms are involved in this effect. Two other observations are in agreement with such a peripheral mechanism. The first one is the chronology of the tacrine-induced bradycardia which occurred before blood pressure increased and disappeared long before the pressor response ended, suggesting that a baroreflex phenomenom is unlikely. There was indeed no parallelism between the temporal profile of the acetylcholinesterase inhibitors-induced pressor and bradycardic responses, further supporting the dissociation of the mechanisms mediating both effects. The second observation is that bradycardia was not altered by the ganglionic blocker chlorisondamine. In fact, chlorisondamine rather enhanced and prolonged the tacrine-induced bradycardia. This phenomenom may be due to the suppression of the tachycardic effects of the sympathetic outflow. Our results thus suggest that tacrine acts on heart rate by potentiating the postganglionic effects of the parasympathetic system inducing bradycardia via M_2 cardiac receptors.

In conclusion, the present data demonstrate that acetylcholinesterase inhibitors induce significant cardiovascular effects in the conscious rat. These effects consist of a long lasting increase in blood pressure involving the central cholinergic system via mainly M_2 and partially M_1 receptors and a bradycardia involving the potentiation of the peripheral parasympathetic cardiac effects. New compounds, like rivastigmine seem to have a lower propensity to induce blood pressure changes, in the conscious animal, as compared with tacrine and physostigmine. Cardiovascular side effects may be of clinical relevance in humans, specially in patients with Alzheimer's disease, who belong to the elderly population and may suffer from associated hypertension and mixed, degenerative and vascular dementia. Clinical comparative and pharmacovigilance studies will be necessary to confirm, in treated patients, if the new generation of acetylcholinesterase inhibitors actually induce fewer cardiovascular side effects than the older ones.

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