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Respective contributions of α-adrenergic and non-adrenergic mechanisms in the hypotensive effect of imidazoline-like drugs

[1,5]Véronique Bruban, [1,5]Josiane Feldman, [1]Hugues Greney, [1]Monique Dontenwill, [1]Stephan Schann, [2]Christian Jarry, [3]Marc Payard, [4]Jean Boutin, [4]Elizabeth Scalbert, [4]Bruno Pfeiffer, [4]Pierre Renard, [4]Paul Vanhoutte & *,[1]Pascal Bousquet

[1]Laboratoire de Neurobiologie et Pharmacologie Cardiovasculaire, Faculté de Médecine, Université Louis Pasteur, 11 rue Humann, 67000 Strasbourg, France; [2]Laboratoire de Chimie Physique, Faculté des Sciences Pharmaceutiques, Université Bordeaux 2, 146 rue Leo Saignat, 33076 Bordeaux cedex, France; [3]Laboratoire de Chimie Pharmaceutique, Faculté de Pharmacie, 35 chemin des Maraîchers, 31400 Toulouse, France and [4]Institut de Recherches Internationales Servier, 6 place des Pléiades, 92415 Courbevoie cedex, France

1 The hypotensive effect of imidazoline-like drugs, such as clonidine, was first attributed to the exclusive stimulation of central α_2-adrenoceptors (α_2ARs).

2 However, a body of evidence suggests that non-adrenergic mechanisms may also account for this hypotension.

3 This work aims (i) to check whether imidazoline-like drugs with no α_2-adrenergic agonist activity may alter blood pressure (BP) and (ii) to seek a possible interaction between such a drug and an α_2ARs agonist α-methylnoradrenaline (α-MNA).

4 We selected S23515 and S23757, two imidazoline-like drugs with negligible affinities and activities at α_2ARs but with high affinities for non-adrenergic imidazoline binding sites (IBS).

5 S23515 decreased BP dose-dependently ($-27\pm5\%$ maximal effect) when administered intracisternally (i.c.) to anaesthetized rabbits. The hypotension induced by S23515 (100 μg kg^{-1} i.c.) was prevented by S23757 (1 mg kg^{-1} i.c.) and efaroxan (10 μg kg^{-1} i.c.), while these compounds, devoid of haemodynamic action by themselves, did not alter the hypotensive effect of α-MNA (3 and 30 μg kg^{-1} i.c.). Moreover, the α_2ARs antagonist rauwolscine (3 μg kg^{-1} i.c.) did not prevent the effect of S23515.

6 Finally, whilst 3 μg kg^{-1} of S23515 or 0.5 μg kg^{-1} of α-MNA had weak hypotensive effects, the sequential i.c. administration of these two drugs induced a marked hypotension ($-23\pm2\%$).

7 These results indicate that an imidazoline-like drug with no α_2-adrenergic properties lowers BP and interacts synergistically with an α_2ARs agonist.

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Abbreviations: α-MNA, α-methylnoradrenaline; α_2ARs, α_2-adrenoceptors; BP, blood pressure; cyclic AMP, cyclic 5′-adenosine monophosphate; GTPγS, guanosine 5′-O-(3-thio)triphosphate; i.c., intracisternally; IBS, non-adrenergic imidazoline binding sites; MAP, mean arterial pressure; NA, noradrenaline; RVLM, rostroventrolateral reticular nucleus

Introduction

Implication of α-adrenoceptors (αARs) in the central hypotensive effect of imidazoline drugs, such as clonidine, has been demonstrated by the use of specific antagonists (Schmitt & Fenard, 1973). It was then specified that α_2ARs was the type involved in this effect. Indeed, the antagonists used to prevent or reverse the effects of clonidine-like drugs are selective for α_2ARs (Hieble & Kolpak, 1993; Timmermans *et al*., 1981). Recently, MacMillan *et al*. (1996) showed that α_2-adrenergic agonists reduce blood pressure (BP) by acting on the α_{2A}ARs subtype.

Alternatively, other groups have reported experimental data suggesting that the exclusive implication of α_2ARs in the hypotensive effect of clonidine-like drugs is unlikely. Within the brainstem, the rostroventrolateral reticular nucleus (RVLM) which contains cardiovascular sympathoexcitatory premotor neurons has been established as the primary site of the hypotensive action of imidazoline-like drugs (Bousquet *et al*., 1981). Direct administration of α-adrenergic agonists with phenylethylamine structures into this region did not mimic the effects of imidazoline drugs (Bousquet *et al*., 1984; Ernsberger *et al*., 1990). In addition, α_2ARs antagonists failed to prevent imidazolines-induced hypotension when administered directly in the RVLM. On the contrary, microinjection of antagonists with imidazoline structures, such as idazoxan and efaroxan, prevented the action of clonidine analogues (Chan & Head, 1996; Haxhiu *et al*., 1994; Feldman *et al*., 1990). The assumption that there are non-adrenergic receptors sensitive to imidazolines was based on these data. Since then, binding studies have suggested the existence of specific binding sites for imidazoline compounds, which are

*Author for correspondence;
E-mail: Pascal.Bousquet@medecine.u-strasbg.fr.
[5]The first two authors contributed equally to this work.

insensitive to catecholamines (Bricca *et al*., 1989; Meeley *et al*., 1986). These imidazoline binding sites (IBS) have been classified in two main subtypes (Michel & Insel, 1989). The imidazoline I_1 binding sites (I_1BS) have high affinity for clonidine and idazoxan whereas imidazoline I_2 binding sites (I_2BS) are sensitive to idazoxan but not to clonidine. Whether or not I_1BS might be involved in non-adrenergic mechanism underlying the hypotensive effect of imidazoline-like drugs remains questionable. This was, at least in part, the consequence of the use of hybrid pharmacological tools binding both to α_2ARs and to IBS. Newly available selective non-adrenergic substances, able to recognize imidazoline binding sites only, should help to clarify the respective contributions of α_2-adrenergic and non-adrenergic mechanisms in the central hypotensive effects of imidazoline-like drugs. The aim of this study was (i) to investigate whether imidazoline-like drugs with no α_2-adrenergic properties could modify BP after central injection and (ii) to determine whether α_2-adrenergic and non-adrenergic drugs may interact synergistically to lower BP.

Methods

Radioligand binding assays

I_1-binding sites Bovine adrenal medullary plasma membranes were prepared as described (Molderings *et al*., 1993). Membranes (0.8 mg protein ml^{-1}) were incubated for 40 min with 7 nM [^{3}H]-clonidine at 22°C in binding buffer ((mM) PBS, EGTA 0.5, $MgCl_2$ 0.5, 0.5% ascorbic acid, pH 7.5) and increasing concentrations of competitors (10^{-9} to 10^{-4} M) in the presence of 1 μM RX821002 to mask α_2ARs. Non-specific binding was defined as [^{3}H]-clonidine binding in the presence of 1 μM of S22687 (which appears as a high affinity I_1 competing drug, $K_i = 4.98$ nM).

I_2-binding sites Rabbit kidney membranes preparation and affinities of drugs were performed as described (Pigini *et al*., 1997) except that 2-BFI (10 μM) was used to define non-specific binding instead of cirazoline (10 μM).

α_1- and α_2-adrenoceptors binding assays Calf frontal cortex membranes were prepared as described (Van Liefde *et al*., 1993) for binding assays to α_1- and α_2ARs. α_1-adrenergic binding: membranes (0.5 mg protein ml^{-1}) were incubated for 40 min at 25°C with 0.5 nM [^{3}H]-prazosin in 50 mM phosphate buffer, 10 mM $MgCl_2$, and increasing concentrations of competitors (10^{-9} to 10^{-4} M) in a final volume of 525 μl. α_2-adrenergic binding: membranes (0.5 mg protein ml^{-1}) were incubated for 60 min at 25°C with 0.8 nM [^{3}H]-RX821002 in the presence of 0.3 μM serotonin to mask $5HT_{1A}$ receptors in 50 mM sodium phosphate buffer, pH 7.4, with increasing concentrations of competitors (10^{-9} to 10^{-4} M). Non-specific binding was defined with 10 μM phentolamine in both assays. The remaining protocol is described elsewhere (Greney *et al*., 2000).

Intracellular cyclic AMP assay

HT29 cells were grown in DMEM (high glucose) with 10% foetal calf serum in an 8% CO_2 incubator. After 48 h culture without serum, cells were harvested by mild trypsination in DMEM – 10% FBS and pelleted at $500 \times g$ for 5 min. Cells were washed twice with DMEM and brought up to 10^6 cells ml^{-1} in DMEM containing 50 mM HEPES and 250 μM isobutylmethylxanthine. From this cell suspension, 10^5 cells/tube were incubated in a total volume of 200 μl at 37°C for 15 min with 5 μM forskolin in the presence or absence of drugs. The remaining protocol is described elsewhere (Greney *et al*., 2000).

$GTP_\gamma[^{35}S]$ binding assay

Methods described by Jasper *et al*. (1998) were modified as follows. Cell pellets of human α_{2A}AR transfected CHO cells (clone 1E5) were suspended in homogenization buffer (HEPES/NaOH 20 mM, pH 7.4) and lysed using a Polytron. Homogenate was centrifuged at 23,000 r.p.m. for 30 min at 4°C and the supernatant was removed. The pellet was resuspended in HEPES/NaOH 20 mM, pH 7.4 homogenized with a Potter and sonicated for 15 s. Membrane aliquots were frozen at −80°C. Membranes were thawed and diluted with buffer (HEPES/NaOH 20 mM, NaCl 100 mM, $MgCl_2$ 3 mM, GDP 3 μM, pH 7.4) to 0.04 mg protein ml^{-1}. After a preincubation in buffer for 30 min, 150 μl membranes were incubated with 0.2 nM [^{35}S]-$GTP_\gamma S$ and drugs for 1 h at 25°C. Non-specific binding was defined by 10 μM cold $GTP_\gamma S$. Reactions were stopped by vacuum filtration over GF/B filters. Filters were washed with ice-cold buffer (HEPES/NaOH 20 mM, NaCl 100 mM, $MgCl_2$ 3 mM, pH 7.4 at 4°C); incorporated radioactivity was determined using liquid scintillation counting.

Animals and haemodynamic measurements

This work was conducted in compliance with Institutional Guidelines and those formulated by the European Community for use of experimental animals (L358 to 86/609/EEC). Normotensive male rabbits (Zika strain) weighing 2.5 to 3.5 kg were prepared as described elsewhere (Feldman *et al*., 1990) except doses of pentobarbitone (40 mg kg^{-1}) and pancuronium bromide (1 mg kg^{-1}). Mean arterial pressure (MAP) was calculated as diastolic pressure plus one third of the differential pressure. The heart rate (HR) was continuously monitored from the pressure signal with a Gould Biotach amplifier (model 13-4615-66).

Intracisternal injections

The animal's head was placed in a stereotaxic frame (La Précision Cinématographique Française). At the beginning of each experiment, a volume of cerebrospinal fluid equal to the one injected during the experiment was withdrawn. The volume of i.c. injections was 100 μl except for S23757 (400 μl). In antagonism studies, as well as in synergistic experiments, there was an interval of 10 to 15 min between the two injections of drugs.

Drugs and supplies

Sodium pentobarbitone (Sanofi, Libourne, France), pancuronium bromide (Pavulon, Organon Teknika, Fresnes, France), (−)-α-methylnorepinephrine, (−)-norepinephrine

bitartrate, and clonidine (RBI-Bioblock, Strasbourg, France), [^{35}S]-GTP$_\gamma$S (1100 Ci mmol^{-1}), [^{3}H]-clonidine (61.9 Ci mmol^{-1}), and [^{3}H]-prazosin (77.9 Ci mmol^{-1}) (NEN, Paris, France), [^{3}H]-idazoxan (45 Ci mmol^{-1}) and [^{3}H]-RX821002 (59 Ci mmol^{-1}) (Amersham Pharmacia Biotech, Orsay, France), efaroxan, rauwolscine, and forskolin (Sigma Chemical, L'Isle d'Abeau Chesnes, France), S23515: (±)-5-(2-bromophenoxy) methyl-2-amino-4, 5-dihydro-1, 3 oxazole, S23757: (±)-2-(2-fluoro-5-methylphenyl)-4,5-dihydro-1H-imidazole and S22687: (±)-5-(2-methylphenoxy)methyl-2-amino-4,5-dihydro-1,3 oxazole) were kindly provided by the Institut de Recherches Internationales Servier (Courbevoie, France), MK912 was a gift from Merck Sharp & Dohme Research Laboratories (U.S.A.). Drugs were dissolved in saline solution and pH was adjusted to 7.4.

HT29 cells were kindly provided by Dr H. Paris (Toulouse, France) and CHO cells by Dr A.D. Strosberg (Paris, France).

Statistics and calculation

Data are given as mean ± s.e.mean or s.e. Homogeneity of initial cardiovascular parameters between groups was checked with an ANOVA. Mean values were compared with an ANOVA (for repeated measures or inter-group) or Student *t*-tests (paired or unpaired observations) as appropriate. *P* values <0.05 were used as the level of significance, *n* was the number of experiments.

Results

Radioligand binding assays

S23515, as rilmenidine, is an aminooxazoline compound and S23757 is an imidazoline close to benazoline and idazoxan (Figure 1) (Pigini *et al*., 1997). They were highly selective for I_1BS over α_1- and α_2ARs (Table 1). Moreover, S23515 was selective for I_1BS over I_2BS. We further investigated affinity of S23515 for other receptors (5HT, dopamine, NMDA, $GABA_A$, $GABA_B$, histamine, NPY, opioids, cannabinoids, muscarinic, nicotinic) and channels (Ca^{2+} L-type, K^+ voltage- and ATP-dependant); K_i values were always $>10^{-5}$ M (data not shown).

Intracellular cyclic AMP assay

In HT29 cells, clonidine inhibited forskolin-stimulated cyclic AMP production with an $IC_{50} = 62.5 \pm 16.4$ nM whereas S23515 failed to do so, demonstrating its lack of agonist activity at α_{2A}ARs (Figure 2). S23515 (1 μM) did not antagonize clonidine's effect (data not shown).

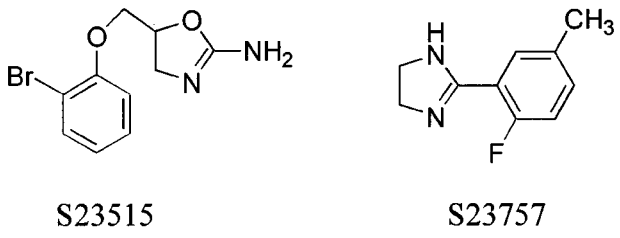

Figure 1 Chemical structures of S23515 and S23757.

GTPγ[^{35}S]-binding assay

In CHO cells transfected with human α_{2A}ARs, noradrenaline (NA) induced [^{35}S]-GTPγS binding to G proteins ($EC_{50} = 709 \pm 113$ nM, Figure 3a). MK 912, an α_2ARs antagonist (Pettibone *et al*., 1987), had no effect by itself but antagonized in a concentration-dependent manner the effect of NA (10 μM) with an IC_{50} value of 30.3 ± 11.7 nM (Figure 3a,b). In this model, S23515 and S23757 had neither agonist nor antagonist effects since they never induced [^{35}S]-GTPγS binding and did not prevent the effect of NA (10 μM) on [^{35}S]-GTPγS binding (Figure 3a,b).

Cardiovascular effects of S23515 and S23757

Intravenous injections (10, 100, 1000 μg kg^{-1}) of S23515 to rabbits did not significantly alter BP and HR (data not shown). However, cumulative i.c. doses of S23515 (10–300 μg kg^{-1}) dose-dependently decreased MAP and HR: 70 ± 5 mmHg vs

Table 1 Affinity of S23515 and S23757 for α_1 and α_2-adrenoceptors and for imidazoline I_1 and I_2 binding sites

	K$_i$ (nM)			
	α_1ARs[a]	α_2ARs[b]	I_1BS[c]	I_2BS[d]
S23515	1,710 ± 474	> 10,000	6.40 ± 1.94	403 ± 112
S23757	> 10,000	> 10,000	5.30 ± 1.48	6.20 ± 1.83

Competition studies were performed on calf frontal cortex membranes labelled with 0.5 nM of [^{3}H]-prazosin[(a)], 0.8 nM of [^{3}H]-RX821002[(b)] for α-adrenergic binding, on bovine adrenal medullary plasma membranes labelled with 7 nM [^{3}H]-clonidine[(c)] and on rabbit kidney membranes (cortex) in presence of 5 nM of [^{3}H]-idazoxan[(d)] for imidazoline binding. K_i values of at least four experiments, each performed in triplicate. Results are given as mean ± s.e.mean. Competition curves were analysed using the iterative non-linear least-squares curve fitting program GraphPad. K_i values were determined using the method of Cheng and Prussof.

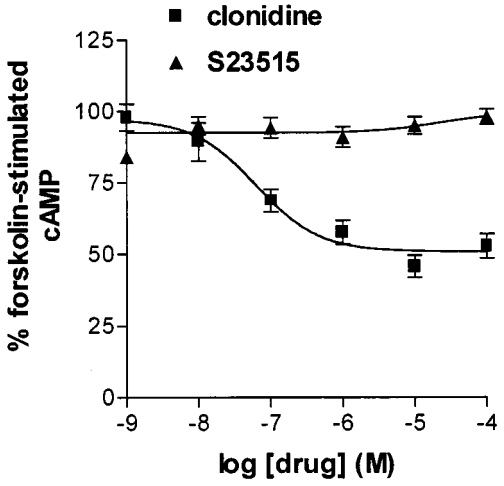

Figure 2 Agonist activity of S23515 and clonidine on α_{2A}ARs in HT29 cells. The intracellular cyclic AMP production was stimulated by forskolin 5 μM in the absence (100% of the response) or in the presence of increasing concentrations of clonidine or S23515. Data are mean ± s.e.mean of three experiments performed in triplicate. Curves were analysed using the iterative non-linear least-squares curve fitting program GraphPad.

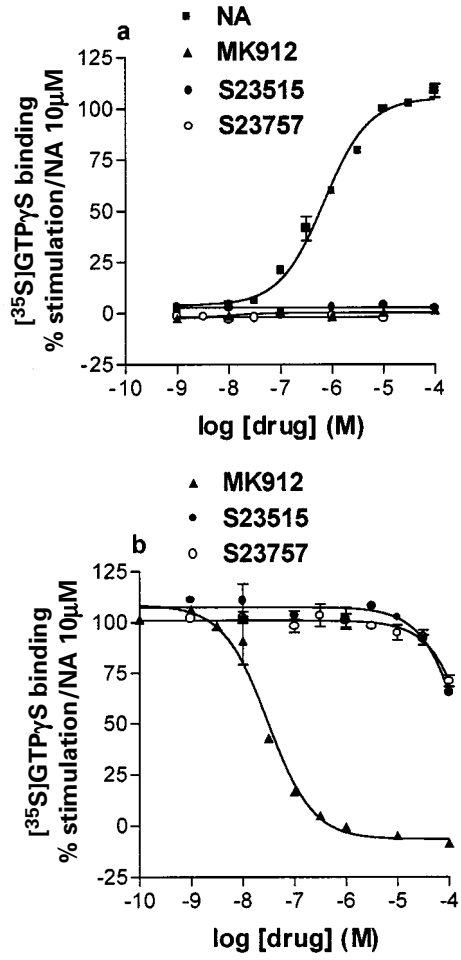

Figure 3 α_{2A}ARs-mediated stimulation of [^{35}S]-GTPγS binding to G proteins in CHO cell membranes expressing the human α_{2A}ARs. Membranes were incubated with [^{35}S]-GTPγS (0.2 nM) in the presence of different drugs. (a) Agonist activity: concentration-response curves of NA, MK912, S23515, S23757 (b) Antagonist activity: effect of NA (10 μM) in presence of increasing concentrations of MK912, S23515, or S23757. Results are expressed as a percentage of the response induced by 10 μM of NA. Data are mean$\pm$s.e.mean. The curves are representative of three experiments performed in triplicate.

96 ± 2 mmHg (Figure 4) and 243 ± 14 beats min^{-1} vs 290 ± 11 beats min^{-1}, respectively, at 300 μg kg^{-1}. The hypotension was significant from the dose of 30 μg kg^{-1} onwards and the bradycardia from the dose of 10 μg kg^{-1} onwards ($P<0.05$, $n=6$). Repeated i.c. injections of vehicle did not modify haemodynamic parameters significantly (Figure 4).

S23757 had no significant cardiovascular effects either after i.v. injections (10 μg kg^{-1} to 3 mg kg^{-1}, data not shown) or within the 45 min of i.c. administration of 1 mg kg^{-1} (97 ± 3 mmHg vs 98 ± 3 mmHg, $n=6$). We therefore investigated its potential antagonist activity towards the central hypotensive action of S23515. S23515 (100 μg kg^{-1}) injected i.c. decreased MAP maximally by $26\pm3\%$ (75 ± 5 mmHg vs 100 ± 4 mmHg; $P<0.05$, $n=6$) (Figure 5). In another group of animals treated with S23757 i.c. (1 mg kg^{-1}) prior to the i.c. injection of S23515 (100 μg kg^{-1}), the hypotension was significantly prevented since MAP decreased by $8\pm2\%$ only: from 98 ± 3 to 89 ± 4 mmHg ($n=6$) (Figure 5).

In contrast, the same dose of S23757 did not affect the hypotension induced by two different doses of the α_2ARs agonist α-MNA (3 and 30 μg kg^{-1}, i.c.) (Figure 5). We performed the same experiments using reference imidazoline and α_2ARs antagonists, efaroxan (Haxhiu *et al*., 1994) and rauwolscine, respectively. The effect of S23515 (100 μg kg^{-1}, i.c.) was completely prevented by the imidazoline antagonist efaroxan (10 μg kg^{-1}, i.c.) since MAP varied from 96 ± 3 to 94 ± 4 mmHg ($n=6$) but not by rauwolscine (3 μg kg^{-1}, i.c.). MAP still decreased from 98 ± 3 to 81 ± 2 mmHg ($P<0.05$, $n=6$).

Interaction between imidazoline and α_2-adrenergic drugs

To examine a possible synergism between imidazoline and α_2-adrenergic drugs, we selected subthreshold (S23515) and threshold (α-MNA) doses of the drugs. At 3 μg kg^{-1} i.c.,

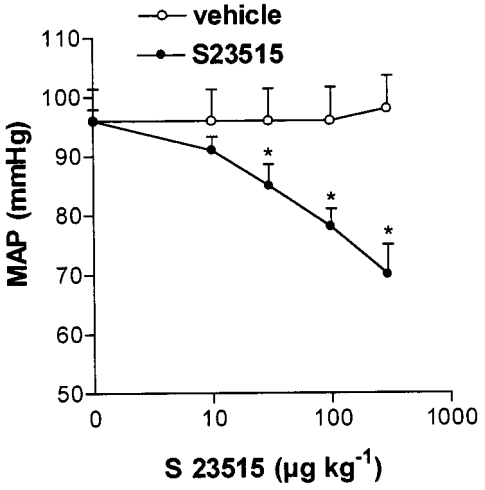

Figure 4 Effect on MAP of cumulative doses of S23515 administered i.c. to anaesthetized rabbits. Repeated injections of vehicle i.c. had no significant effect. Data are mean$\pm$s.e.mean of six experiments for each treatment. *$P<0.05$.

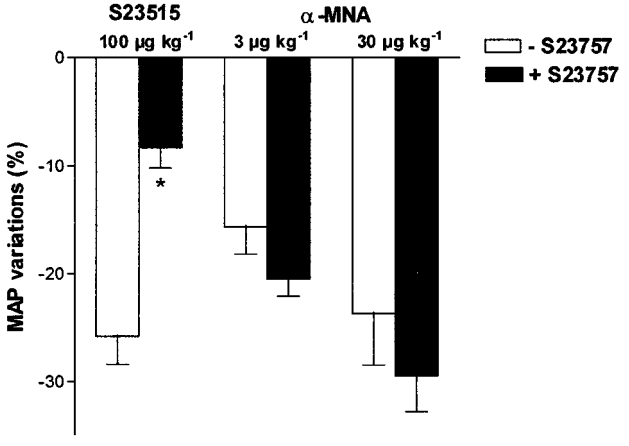

Figure 5 Prevention of the hypotensive actions of S23515 and α-MNA. Vehicle or S23757 (1 mg kg^{-1}) was injected as pretreatment to anaesthetized rabbits. S23515 (100 μg kg^{-1}, $n=6$) or α-MNA (3 and 30 μg kg^{-1}, $n=5$ per group) was subsequently administered. i.c. injections. Data are mean$\pm$s.e.mean. *$P<0.05$.

S23515 had no significant effect on MAP (101±2 mmHg vs 105±3 mmHg, $n=7$) (Figure 6). In another group of rabbits, 0.5 μg kg^{-1} i.c. of α-MNA decreased MAP slightly by 9±1% (91±2 mmHg vs 100±1 mmHg) ($P<0.05$, $n=7$) (Figure 6). In seven other animals, S23515 (3 μg kg^{-1}, i.c.) was given, followed by α-MNA (0.5 μg kg^{-1}, i.c.) 10 min later. Then, the MAP decreased immediately and the maximal effect was reached within 20 min of injection: −23±2% (80±2 mmHg vs 104±3 mmHg) ($P<0.05$). The reduction in MAP in that case was significantly different from that obtained with α-MNA or S23515 alone or with S23515 injected twice at the dose of 3 μg kg^{-1}, i.c. (−10±1 vs −23±2%; $P<0.05$, $n=7$) (Figure 6).

Discussion

The present study shows that S23515, a new imidazoline-like drug devoid of α_2-adrenergic properties, lowers MAP when administered i.c. This effect is prevented by two imidazoline antagonists, S23757 and efaroxan, but not by the reference α_{2A}ARs antagonist rauwolscine. S23515 also appears to interact synergistically with α-MNA to decrease BP.

In binding experiments, S23515 was devoid of affinity for α_2ARs but bound to I_1BS with an affinity in the nM range. It also had negligible affinity for most receptors known to be involved in the central regulation of the vasomotor tone (Sun, 1996). In agreement with the binding data, S23515 did not present any α_{2A}-adrenergic activity either in [^{35}S]-GTPγS binding assays in CHO cells or on intracellular cyclic AMP level in HT29 cells, which express human α_{2A}ARs endogenously but no I_1BS (Greney *et al*., 2000). On the contrary, α-MNA, the reference α_2-adrenergic substance used in our functional experiments, behaves as a full agonist on α_{2A}ARs

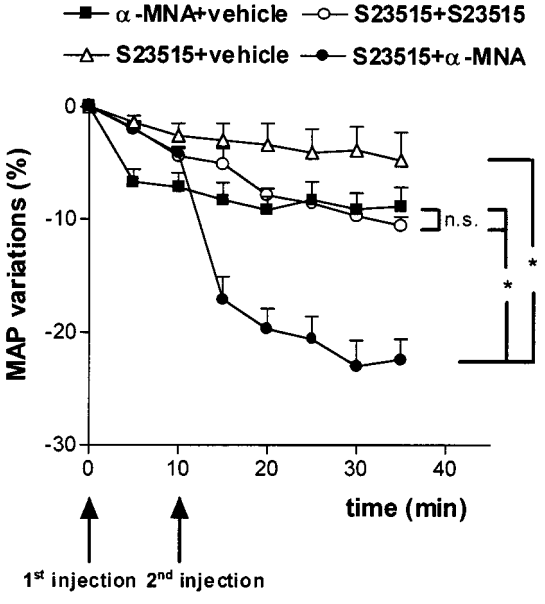

Figure 6 S23515 and α-MNA interact synergistically to decrease MAP. Three groups received S23515 (3 μg kg^{-1}) as first injection, followed by a second injection which consisted of either vehicle or S23515 (3 μg kg^{-1}) or α-MNA (0.5 μg kg^{-1}). The fourth group received α-MNA (0.5 μg kg^{-1}) as first injection followed by an injection of vehicle. i.c. injections.

in [^{35}S]-GTPγS binding assays (Jasper *et al*., 1998). The lack of α-adrenergic properties of S23515 was also supported by the absence of haemodynamic effect after systemic injection to the rabbit.

The central hypotensive action of imidazoline drugs has been largely documented in the rabbit (Head *et al*., 1998; Chan & Head, 1996; Feldman *et al*., 1990). Here we showed that S23515 decreased MAP by acting centrally. The absence of hypotensive action of S23515 after systemic administration revealed, first, that there was no peripheral component in the effect observed when the drug was administered i.c., and second, that it did not cross the blood brain barrier, at least in an active form. Efaroxan has been repeatedly shown to block the hypotensive effect of imidazoline-like drugs (Ernsberger & Haxhiu, 1997) as it did for S23515 in the present study. Nevertheless efaroxan still has some α_2ARs antagonist activity (Berridge *et al*., 1992). Here we report that compound S23757, which had neither affinity nor activity at α_2ARs, also prevented the central hypotensive action of S23515. Interestingly, it did not prevent the effect of α-MNA on BP. As such, it appears as the first antagonist really able to discriminate between the α_2-adrenergic and non-adrenergic mechanisms beyond the sympatho-inhibitory effects of imidazoline-like drugs. Finally, the blockade of the central hypotensive effect of S23515 by S23757 but not by rauwolscine clearly confirm that this action is not mediated by α_2ARs. Our results support the data of Tolentino-Silva *et al*. (2000) showing that moxonidine, another imidazoline-like drug, induces hypotension in D79N mice lacking functional α_{2A}ARs. This however does not corroborate the data of MacMillan *et al*. (1996) obtained in the same strain of mice.

Although rather high doses of S23515 were needed to reduce BP, its binding profile and its sensitivity to S23757 suggest that its hypotensive action might involve I_1BS. In this respect, it is interesting to note that S23515 was 62-time selective for I_1BS over I_2BS.

In the second part of this study, an interaction between α_2-adrenergic and non-adrenergic mechanisms was demonstrated. The sequential i.c. injection of S23515 and of α-MNA, that only induced non-significant or weak decreases of MAP by themselves, caused a marked hypotension, so that there must be a synergistic process between these two mechanisms. Indeed, S23515 injected twice with the same protocol did not lead to such a hypotension. The synergy we observed here could occur between I_1BS of the RVLM and α_{2A}ARs located in the *nucleus tractus solitarii*, the primary site of the hypotensive action of α-MNA (Zandberg *et al*., 1979), and/or in the RVLM. In this context, one can assume that the hypotension induced by imidazoline hybrid drugs, whose site of action is the RVLM (Ernsberger *et al*., 1990; Bousquet *et al*., 1981; 1984), involves such a synergy. S23515 needs higher doses to reduce BP than clonidine or rilmenidine (also an hybrid imidazoline-like drug) do. This might be explained by the absence of such a synergy when using non-α_2-adrenergic imidazoline drugs.

In conclusion, our work establishes that an imidazoline-like drug devoid of α_2-adrenergic properties can modify BP. Our biochemical and functional data suggest that I_1BS might be involved in this non-adrenergic action. Further investigations will be needed to definitively confirm this assumption. In addition, we show that α_2-adrenergic and non-adrenergic mechanisms may interact in a synergistic way to lower BP.

This interaction could be implicated in the regulation of the vasomotor tone as well as in the hypotensive effect of hybrid drugs such as clonidine. S23515 and S23757 represent helpful tools for further studies of imidazolines actions.

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