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Involvement of α_2-adrenoceptors in the cardiovascular effects of moxonidine

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Abstract

The central sympathoinhibition caused by moxonidine has been explained by activation of α_2-adrenoceptors on the one hand, and by an action at imidazoline I_1 receptors on the other hand. In order to examine these possibilities, effects of moxonidine were compared with those of 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK 14304), an α_2-adrenoceptor agonist with very low affinity for I_1 receptors, in conscious rabbits. The interaction with yohimbine, an α_2-adrenoceptor antagonist with very low affinity for imidazoline I_1 receptors, was also studied. Moxonidine 3–100 $\mu g\ kg^{-1}$ and UK 14304 1–30 $\mu g\ kg^{-1}$ i.v. elicited similar effects: they decreased arterial blood pressure after a transient increase, decreased renal sympathetic nerve activity (recorded with chronically implanted electrodes), decreased heart rate and decreased the plasma noradrenaline concentration. Yohimbine given i.v. antagonized the effects of moxonidine and of UK 14304 in a similar manner. Yohimbine injected into the cisterna magna (i.c.) prevented the hypotension but did not change the decrease in plasma noradrenaline and heart rate, again in the case of both moxonidine and UK 14304. The agreement of the effect patterns of moxonidine and UK 14304, and the similar antagonism of yohimbine against either drug, demonstrate involvement of α_2-adrenoceptors in their central sympathoinhibitory action. The resistance of the bradycardia and the plasma noradrenaline fall against yohimbine i.c. indicates a contribution of presynaptic α_2-adrenergic inhibition of transmitter release from postganglionic sympathetic neurons to the overall reduction of sympathetic tone.

Keywords: α_2-Adrenoceptor; Imidazoline receptor; Sympathoinhibition; Presynaptic inhibition; Moxonidine; UK 14304

1. Introduction

Two primary sites of action have been proposed for the central sympathoinhibitory effect of clonidine and related drugs such as rilmenidine and moxonidine: α_2-adrenoceptors (Kobinger, 1986; Armah et al., 1988; Van Zwieten, 1988; Kobinger and Pichler, 1990) and, more recently, 'imidazoline receptors', specifically the imidazoline I_1 receptor in the rostral ventrolateral medulla oblongata (RVLM) (Verbeuren et al., 1990; Bousquet et al., 1992; Chrisp and Faulds, 1992; Molderings et al., 1993; Ernsberger et al., 1993); the RVLM is the main brain region where clonidine-like drugs produce hypotension (Bousquet et al., 1981; reviewed by Philippu, 1988; Verbeuren et al., 1990; Molderings et al., 1993; Dampney, 1994).

The imidazoline receptor hypothesis is mainly based on radioligand binding experiments in which clonidine, rilmenidine and moxonidine possess higher affinity for imidazoline I_1 binding sites than α_2 binding sites. When studied in membranes from the bovine RVLM, the I_1 site/α_2 site affinity ratio was 4 for clonidine and even about 30 for rilmenidine and moxonidine (Ernsberger et al., 1992,1993). Direct evidence for an involvement of I_1 receptors in the effect of clonidine-like drugs, however, is sparse. In the case of moxonidine it is limited, to our knowledge, to a recent study with efaroxan, an α_2-adrenoceptor antagonist with an I_1/α_2 affinity ratio of about 50. Efaroxan, when injected into the RVLM, blocked the hypotensive and bradycardic effect of moxonidine, administered either into the RVLM or i.v. (Haxhiu et al., 1994).

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In two recent studies in conscious rabbits (Szabo et al., 1993; Urban et al., 1994) we examined the interaction of rilmenidine with yohimbine and 6-chloro-*N*-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SK & F 86466), α_2-adrenoceptor antagonists with very low affinity for imidazoline I_1 receptors (Ernsberger et al., 1987,1992; Bricca et al., 1989). Moreover, we compared rilmenidine with 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK 14304), an α_2-adrenoceptor agonist with an I_1 binding site/α_2 binding site affinity ratio < 0.01, about 3000 times lower than for rilmenidine and moxonidine (Ernsberger et al., 1992; Bricca et al., 1993). All drugs were given i.v. The effects of rilmenidine and UK 14304 were qualitatively identical, yohimbine and SK&F 86466 were potent antagonists of rilmenidine, and SK&F 86466 blocked the effects of rilmenidine and UK 14304 to the same extent. The results argued in favour of α_2-adrenoceptors as sites of action of both UK 14304 and rilmenidine (Szabo et al., 1993; Urban et al., 1994).

We have now carried out a similar study with moxonidine. Parameters measured were blood pressure, heart rate, the plasma noradrenaline concentration and, in part of the experiments, renal sympathetic nerve activity. Moxonidine and UK 14304 were given i.v.; yohimbine was given either i.v. or intracisternally (i.c.).

2. Materials and methods

Thirty-three rabbits of mixed breed and either sex were used (1.6–2.6 kg). In one group of animals, moxonidine, UK 14304 and yohimbine were all given i.v. An electrode was chronically implanted at the renal postganglionic sympathetic nerves in these animals, and renal sympathetic nerve activity was recorded in addition to blood pressure, heart rate and plasma noradrenaline. In the second group of animals, moxonidine and UK 14304 were given i.v. but yohimbine was given i.c. A catheter was chronically implanted in the cisterna magna, and only blood pressure, heart rate and plasma noradrenaline were determined.

2.1. Electrode and catheter implantation

An initial operation was carried out under anaesthesia to implant either the renal sympathetic nerve recording electrode or, in other animals, the cisterna magna catheter. Penicillin G 200 000 IU was given i.v. immediately before the operation. Anaesthesia was induced by Saffan (0.5–0.6 ml kg^{-1} i.v.; 1 ml contains 9 mg alfaxolone and 3 mg alfadolone acetate). After tracheal intubation, anaesthesia was continued with halothane 1.5–4.0% in a moistened room air-O_2 mixture. No muscle relaxant was administered, and rabbits breathed spontaneously. Physiological saline 6 ml h^{-1} was infused through an ear vein until the end of the operation.

The sympathetic nerve electrode was implanted as described by Szabo et al. (1993) with exceptions mentioned. The kidney was approached retroperitoneally. The two or three sympathetic nerve trunks accompanying the renal artery were dissected free. A bipolar electrode was prepared from single-strand Teflon-coated stainless steel wire (0.13 mm bare diameter, 0.18 mm coated diameter). The electrode was sutured to the renal artery with atraumatic thread (0.5 metric, DR-9; Serag-Wiessner, Naila, Germany). After the nerve trunks had been slipped into the electrode spirals, nerves and electrode were embedded in silicone gel. The muscle layer was sutured, and a ground electrode from multi-strand Teflon-coated stainless steel wire was sutured to the muscle. The electrode ends were subcutaneously tunneled to an incision in the skin of the neck which was then sutured.

The intracisternal catheter implantation was adopted from Head et al. (1983) with exceptions mentioned. The animal was placed in a stereotaxic frame (Narishige, Tokyo, Japan). The neck muscles were separated in the midline and the atlanto-occipital membrane was exposed. A hole was made through the rostral part of the atlanto-occipital membrane in the midline using a 24 gauge needle. A three-sided pursestring suture had first been sewn around the place of the hole with atraumatic thread. One end of the catheter was inserted through the hole into the cisterna magna. The pursestring suture was tightened and the catheter fixed with two other sutures. The wound was closed and the end of the catheter tunneled to an incision in the neck which was sutured.

2.2. Acute experiments

The first experiment was carried out 2 days after the operation. Further experiments, on average three in one rabbit, followed at intervals of 2–3 days. The animals were killed by an overdose of pentobarbitone (150–200 mg kg^{-1} i.v.) after the last experiment.

The rabbits were sitting in a plastic box (35 × 15 × 15 cm). The root of one ear was infiltrated with 0.1–0.2 ml lidocaine solution 2%. The central ear artery was cannulated with an Abbocath 22 gauge catheter (Abbott, Chicago, IL, USA) for measurement of blood pressure and heart rate and for blood sampling. A marginal ear vein was cannulated with an Abbocath 24 or 26 gauge catheter for administration of drugs. The skin incision in the neck was opened after infiltration with lidocaine and the electrodes or the catheter were recovered.

Arterial blood pressure was measured with a Statham P23Db transducer. Heart rate was integrated from the pressure signal. The arterial plasma concen-

tration of noradrenaline was determined by alumina chromatography followed by high pressure liquid chromatography (Szabo and Schultheiss, 1990); dihydroxybenzylamine 5 ng was added to each sample as internal standard. When the concentration was below the detection limit (twice baseline noise; 10.5 ± 1.4 pg ml^{-1} plasma; $n = 89$), the detection limit was taken as the noradrenaline value (21 from 534 measurements). Adrenaline concentrations were also recorded by the high pressure liquid chromatographic system; they are not included because they were often below the detection limit. In animals with chronically implanted recording electrodes, the frequency of sympathetic spikes was determined using a window discriminator (Szabo et al., 1989).

Treatments and measurements were timed as follows. Animals were first treated with yohimbine (or saline), one dose per experiment, and then with either moxonidine or UK 14304 (or saline), four doses per experiment. The initial yohimbine or saline dose was given either i.v. (injection over 20 s followed by infusion; animals with sympathetic nerve recording) or i.c. (injection over 20 s; no sympathetic nerve recording) 10–30 min after blood vessels had been cannulated and electrode or catheter recovered. Parameters were first evaluated 40 min (yohimbine or saline i.v.) or 10 min (yohimbine or saline i.c.) later ($t = 0$ min in subsequent text), and thereafter every 13 min for a total of 6 times (0, 13 ... 65 min). Blood from the ear artery, 2 ml, was withdrawn at each of the six measurement points; withdrawal took about 3 min and was followed by injection of re-suspended erythrocytes from the preceding withdrawal. Mean arterial pressure, heart rate and renal sympathetic nerve activity were read immediately before blood sampling. Four i.v. injections of either saline or increasing doses of moxonidine or UK 14304 were given briefly after the erythrocyte re-injections of the 2nd to 5th measurement points, so that the 3rd to 6th measurement points represent values about 10 min after the preceding injection. In each experiment, values of the 1st and 2nd measurement points, $t = 0$ and 13 min, were averaged to yield the PRE value, and parameters obtained at the 3rd to 6th measurement points were expressed as a percentage of the PRE value.

The various drug treatment protocols will be detailed in the Results section. No rabbit received a given kind of treatment more than once; the order of treatments in any rabbit was random.

2.3. Statistics

Means ± S.E.M. are presented throughout. PRE values of saline-and yohimbine-treated rabbits were compared with the Mann-Whitney test (Table 1). Effects of moxonidine or UK 14304 were compared to saline, and effects of moxonidine or UK 14304 in saline-pretreated animals were compared to their effects in yohimbine-pretreated animals, by repeated measures analysis of variance, based on the 'percentage of PRE' values ('MANOVA procedure'; SPSS/PC$^+$ Advanced Statistics V4.01 (1991); SPSS, Chicago, IL, USA). The limit of significance was taken as $P < 0.05$, and only this level is indicated even where $P < 0.01$.

2.4. Drugs

These were UK 14304 tartrate (Pfizer, Sandwich, UK), halothane (ICI, Plankstadt, Germany), lidocaine HCl (Astra, Wedel, Germany), penicillin G (Grünenthal, Stolberg, Germany), moxonidine (Beiersdorf, Hamburg, Germany), Saffan ampoules (alfaxalone + alfadolone acetate; Schweizerisches Seruminstitut, Bern, Switzerland), yohimbine hydrochloride (Roth, Karlsruhe, Germany). Drugs were dissolved in saline. Doses refer to salts. Intravenous injections had a volume of 1 ml kg^{-1}, i.v. infusions of 1.92 ml h^{-1}, i.c. injections of 25 μl kg^{-1}.

Table 1
Mean arterial pressure, heart rate, renal sympathetic nerve activity and plasma concentration of noradrenaline

	Pretreatment i.v. with			Pretreatment i.c. with	
	Saline ($n = 20$)	Yohimbine (low dose, $n = 17$)	Yohimbine (high dose, $n = 13$)	Saline ($n = 21$)	Yohimbine ($n = 18$)
Mean arterial pressure (mm Hg)	77.4 ± 2.0	79.1 ± 2.5	73.4 ± 1.3	74.2 ± 2.9	72.8 ± 2.1
Heart rate (min^{-1})	240 ± 6	237 ± 7	243 ± 9	227 ± 7	209 ± 7
Renal sympathetic nerve activity (impulses s^{-1})	21.2 ± 2.4	26.9 ± 4.4	35.4 ± 6.0		
Plasma noradrenaline concentration (pg ml^{-1})	312 ± 29	342 ± 26	538 ± 123	265 ± 22	289 ± 59

Rabbits were pretreated either with saline or yohimbine and either i.v. or i.c. Intravenous pretreatment consisted of an injection of saline 1 ml kg^{-1} followed by an infusion of 1.92 ml h^{-1}, or an injection of yohimbine 0.5 mg kg^{-1} followed by an infusion of 0.1 mg kg^{-1} h^{-1} ('low dose'), or an injection of yohimbine 1 mg kg^{-1} followed by an infusion of 0.2 mg kg^{-1} h^{-1} ('high dose'); injections were given at $t = -40$ min. Intracisternal pretreatment consisted of an injection of saline 25 μl kg^{-1} or an injection of yohimbine 1.5 μg kg^{-1}; injections were given at $t = -10$ min. Values are the PRE values, i.e. averages of the values determined at $t = 0$ and $t = 13$ min.

3. Results

3.1. Pretreatment with saline or yohimbine i.v.

An initial i.v. injection and the subsequent infusion of saline did not overtly affect blood pressure, heart rate and renal sympathetic nerve activity (Fig. 1). Yohimbine was given i.v. either at 0.5 mg kg^{-1} followed by an infusion of 0.1 mg kg^{-1} h^{-1} ('low dose') or 1 mg kg^{-1} followed by an infusion of 0.2 mg kg^{-1} h^{-1} ('high dose'). The bolus injection of yohimbine 0.5 or 1 mg kg^{-1} did not consistently change blood pressure and increased heart rate and renal sympathetic nerve activity, but the latter parameters recovered during the subsequent infusion of yohimbine. At $t = 0$ and 13 min, when the PRE values were read, there was no difference between saline and the two yohimbine groups (Table 1). The values in saline-pretreated rabbits are similar to those obtained previously (Urban et al., 1994).

All parameters remained stable when saline 1 ml kg^{-1} was injected i.v. after the 13-, 26-, 39-and 52-min measurement points. This was true in animals that had initially received saline ($n = 7$) as well as in those pretreated with the low ($n = 5$) or high dose ($n = 2$) of yohimbine (not shown).

Moxonidine 3–100 μg kg^{-1}, when injected into saline-pretreated rabbits, had a biphasic effect on blood pressure: transient hypertension followed by prolonged hypotension (Fig. 1). Both phases of the response were dose-dependent (Fig. 1); the maximal decrease as evaluated at the next measurement point, 10 min after the injection, averaged 14% (Fig. 2A). Heart rate also was dose-dependently reduced (Fig. 1 and Fig. 3A), and renal sympathetic nerve activity was progressively diminished and finally abolished (Fig. 1 and Fig. 4A; the remaining apparent activity in Fig. 1 is due to noise). The noradrenaline plasma concentration was reduced by up to 76% (Fig. 5A).

Similar results were obtained with UK 14304 1–30 μg kg^{-1} in saline-pretreated rabbits: a biphasic blood pressure change, both phases of which were dose-related, with a maximal decrease by 18% (Fig. 2B); bradycardia ($P > 0.05$; Fig. 3B); a depression and finally complete suppression of renal sympathetic nerve activity (Fig. 4B); and a fall of the plasma noradrenaline level, by 95% at the 30 μg kg^{-1} dose (Fig. 5B). Overall, the effects of UK 14304 resembled those obtained in a previous study under the same conditions (Urban et al., 1994).

Pretreatment with the low or the high dose of yohimbine attenuated the initial hypertensive effect (not shown) as well as the inhibitory effects of moxonidine (Fig. 2A, Fig. 3A, Fig. 4A and Fig. 5A). The antagonism was more marked with the high dose except in the case of the plasma noradrenaline level (Fig. 5A).

Pretreatment with either dose of yohimbine also

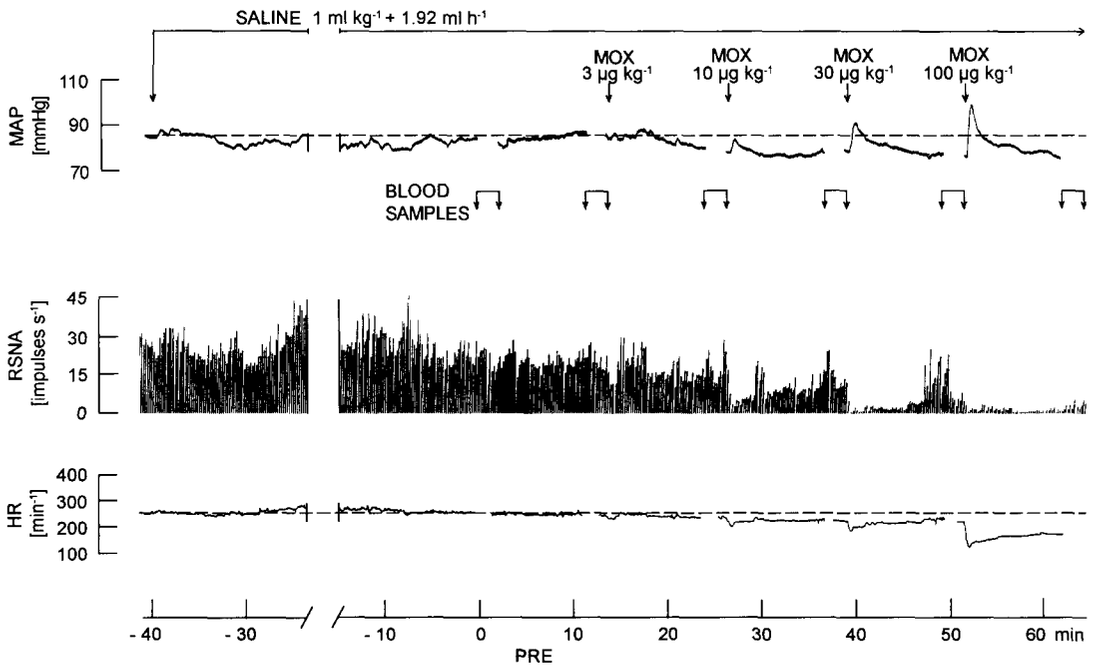

Fig. 1. Mean arterial pressure (MAP), renal sympathetic nerve activity (RSNA) and heart rate (HR) in an experiment with moxonidine in a rabbit pretreated with saline 1 ml kg^{-1} *i.v.* followed by an infusion of saline 1.92 ml h^{-1}. Blood samples were taken and moxonidine (MOX) was administered i.v. as indicated. Blood pressure and heart rate were not measured during blood sampling. The 0-min and 13-min measurement points were used in each experiment to calculate the PRE values (dashed lines).

attenuated the initial hypertensive effect (not shown) as well as the depressive effects of UK 14304 (Fig. 2B, Fig. 3B, Fig. 4B and Fig. 5B). In the case of blood pressure and sympathetic nerve activity, the antagonism was more marked for the high than for the low dose. In the case of the plasma noradrenaline level, the dose-response curves of UK 14304 after the low and the high yohimbine dose, like the dose-response curves of moxonidine, were closely similar (Fig. 5B). The antagonism of yohimbine against the bradycardic effect

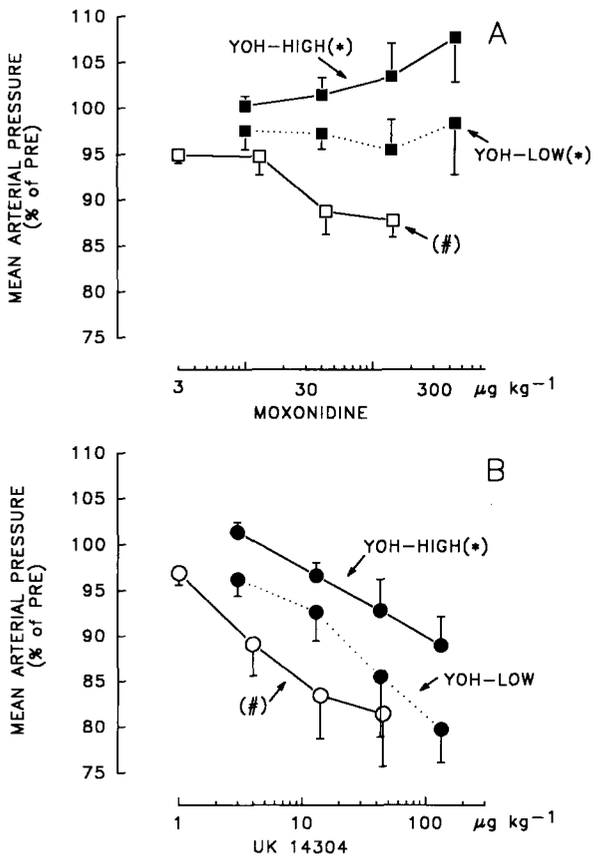

Fig. 2. Effect of (A) moxonidine and (B) UK 14304 on mean arterial pressure in rabbits pretreated *i.v.* with saline (empty symbols), a low dose of yohimbine (filled symbols, YOH-LOW) or a high dose of yohimbine (filled symbols, YOH-HIGH). Protocol illustrated in Fig. 1. Mean arterial pressure was read about 10 min after injection of the respective dose of agonist (at next measurement point) and is expressed as a percentage of PRE (ordinates). Pretreatment dose of saline was 1 ml kg^{-1} followed by 1.92 ml h^{-1}. Low yohimbine dose was 0.5 mg kg^{-1} followed by 0.1 mg kg^{-1} h^{-1}, high yohimbine dose 1 mg kg^{-1} followed by 0.2 mg kg^{-1} h^{-1}. Injected doses (i.v.) of moxonidine were 3, 10, 30 and 100 μg kg^{-1} in saline-pretreated and 10, 30, 100 and 300 μg kg^{-1} in yohimbine-pretreated rabbits. Injected doses (i.v.) of UK 14304 were 1, 3, 10 and 30 μg kg^{-1} in saline-pretreated and 3, 10, 30 and 90 μg kg^{-1} in yohimbine-pretreated rabbits. Abscissae show *cumulative* doses. Means ± S.E.M., $n = 5–7$. # Significant difference ($P < 0.05$) from saline-pretreated rabbits given four injections of saline instead of agonist. * Significant difference ($P < 0.05$) from agonist effect in saline-pretreated rabbits.

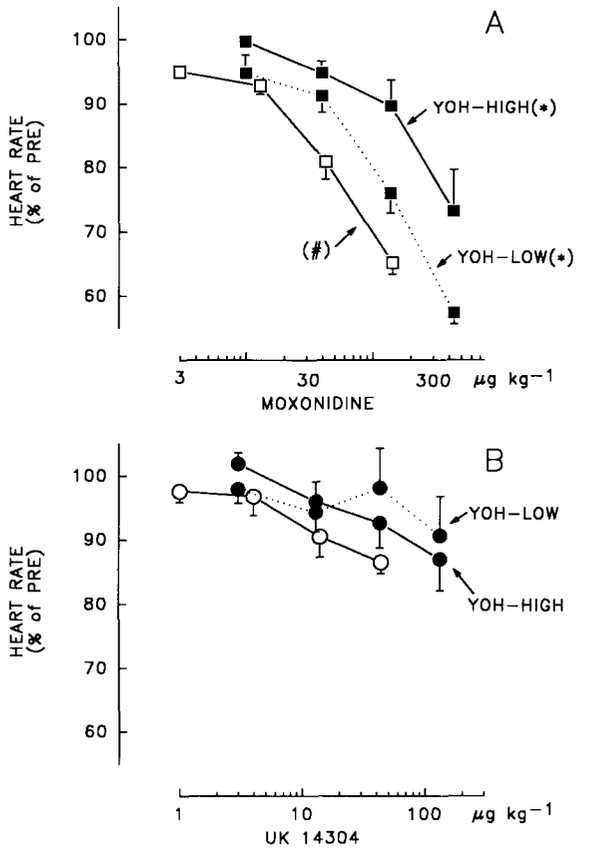

Fig. 3. Effect of (A) moxonidine and (B) UK 14304 on heart rate in rabbits pretreated *i.v.* with saline (empty symbols), a low dose of yohimbine (filled sympols; YOH-LOW) or a high dose of yohimbine (filled symbols; YOH-HIGH). Heart rate was read about 10 min after injection of the respective dose of agonist (at next measurement point) and is expressed as a percentage of PRE (ordinates). See legend to Fig. 2 for details.

of UK 14304 did not reach statistical significance, presumably because the bradycardia was small and non-significant anyway (Fig. 3B).

3.2. Pretreatment with saline or yohimbine i.c.

An initial i.c. injection of saline 25 μl kg^{-1} or yohimbine 1.5 μg kg^{-1} i.c. (Fig. 6) did not consistently affect blood pressure and heart rate. There was no significant difference between the two groups at $t = 0$ and 13 min, when the PRE values were read (Table 1).

All parameters remained stable when saline 1 ml kg^{-1} was injected i.v. after the 13-, 26-, 39-and 52-min measurement points, both in rabbits pretreated i.c. with saline ($n = 6$) and in those pretreated with yohimbine ($n = 6$) (not shown).

The effects of moxonidine 3–100 μg kg^{-1} and UK 14304 1–30 μg kg^{-1} i.v. in rabbits pretreated with saline i.c. were as in rabbits pretreated with saline i.v.

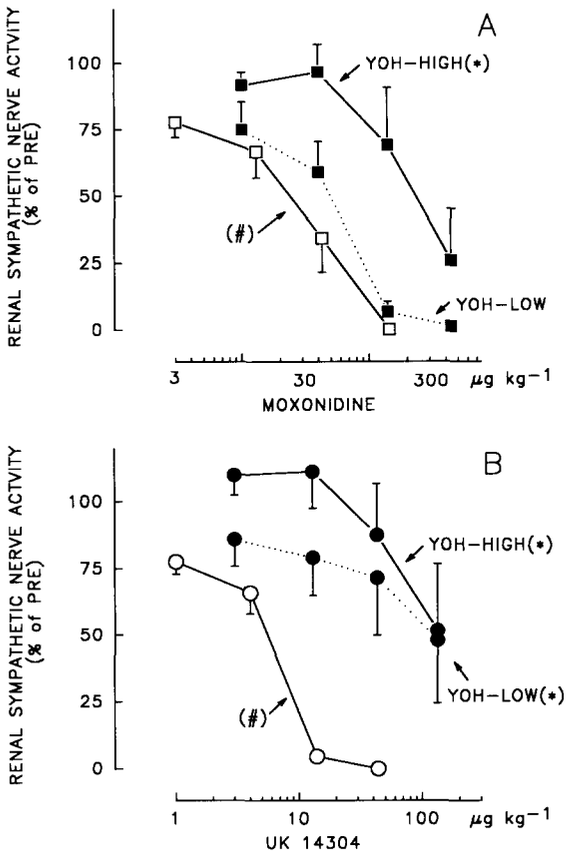

Fig. 4. Effect of (A) moxonidine and (B) UK 14304 on renal sympathetic nerve activity in rabbits pretreated *i.v.* with saline (empty symbols), a low dose of yohimbine (filled symbols; YOH-LOW) or a high dose of yohimbine (filled symbols; YOH-HIGH). Renal sympathetic nerve activity was read about 10 min after injection of the respective dose of agonist (at next measurement point) and is expressed as a percentage of PRE (ordinates). See legend to Fig. 2 for details.

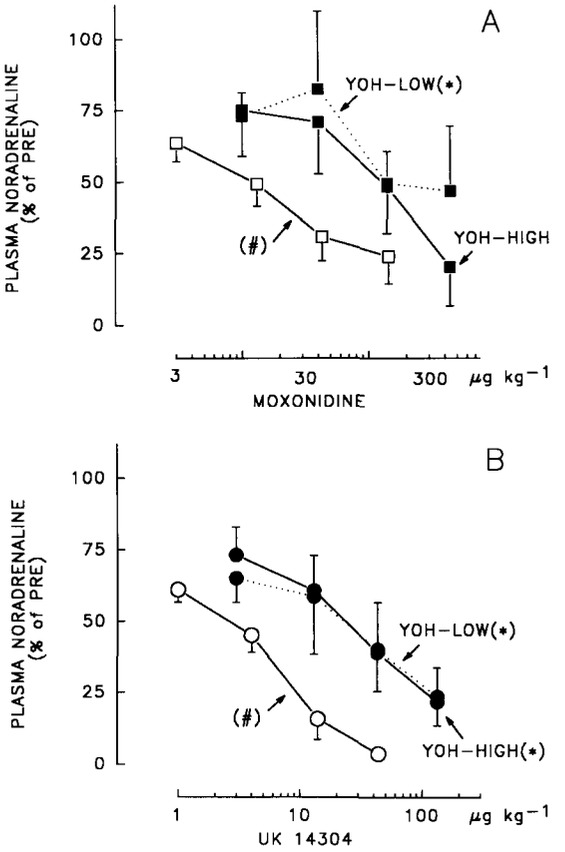

Fig. 5. Effect of (A) moxonidine and (B) UK 14304 on the plasma noradrenaline concentration in rabbits pretreated *i.v.* with saline (empty symbols), a low dose of yohimbine (filled symbols; YOH-LOW) or a high dose of yohimbine (filled symbols; YOH-HIGH). Plasma for noradrenaline determination was obtained about 10 min after injection of the respective dose of agonist (at next measurement point) and the level is expressed as a percentage of PRE (ordinates). See legend to Fig. 2 for details.

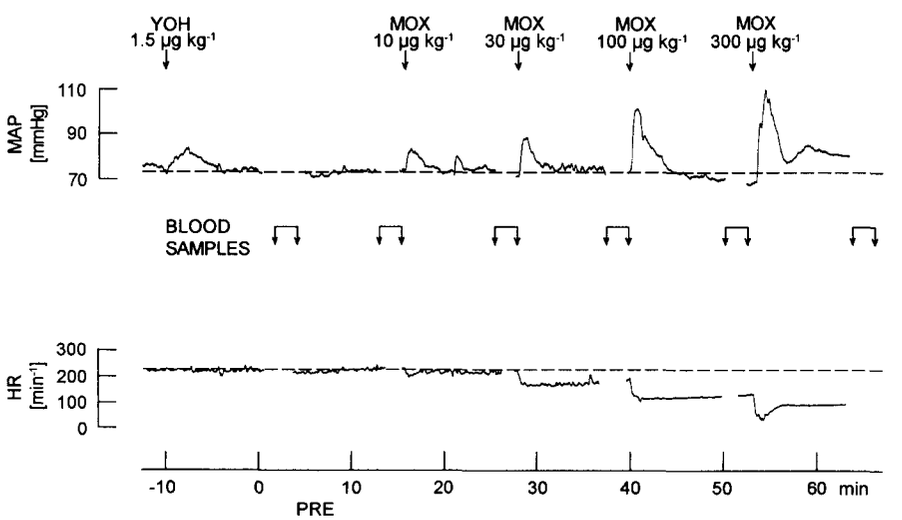

Fig. 6. Mean arterial pressure (MAP) and heart rate (HR) in an experiment with moxonidine in a rabbit pretreated with yohimbine (YOH) 1.5 μg kg^{-1} *i.c.* Blood samples were taken and moxonidine (MOX) was administered i.v. as indicated. Blood pressure and heart rate were not measured during blood sampling. The 0-min and 13-min measurement points were used in each experiment to calculate the PRE values (dashed lines).

(Fig. 7, Fig. 8 and Fig. 9). The maximal hypotension caused by moxonidine amounted to 8% and the fall in the plasma noradrenaline level after moxonidine 100 μg kg^{-1} to 90%. The maximal hypotension caused by UK 14304 after i.c. saline amounted to 16% and the plasma noradrenaline decrease after the 30 μg kg^{-1} dose to 79%. UK 14304 produced significant bradycardia in this series.

The antagonism of i.c. yohimbine against monoxidine partly differed from, and partly agreed with, the antagonism by i.v. yohimbine. In contrast to i.v. yohimbine, i.c. yohimbine did not attenuate the initial blood pressure rise caused by moxonidine, but in agreement with i.v. yohimbine, i.c. yohimbine abolished the subsequent hypotensive phase (Fig. 6 and Fig. 7A). The highest dose of moxonidine actually caused a long-lasting increase in mean arterial pressure after i.c. yohimbine (Fig. 6 and Fig. 7A). Again in contrast to i.v. yohimbine, i.c. yohimbine did not change the fall in heart rate (Fig. 6 and Fig. 8A) and plasma noradrenaline (Fig. 9A) caused by moxonidine. Comparison of Fig. 6 with Fig. 1 shows the persistence of the initial blood pressure peak, the blockade of the hypotension, and the persistence of the bradycardia after yohimbine i.c. (note higher moxonidine doses in Fig. 6).

Yohimbine i.c. also differed partly from, and agreed partly with, i.v. yohimbine in its effect against UK 14304. As in the case of moxonidine, the initial hypertensive phase persisted (not shown) whereas the hypotension was blocked (Fig. 7B). Moreover, as in the case of moxonidine, yohimbine i.c. did not antagonize

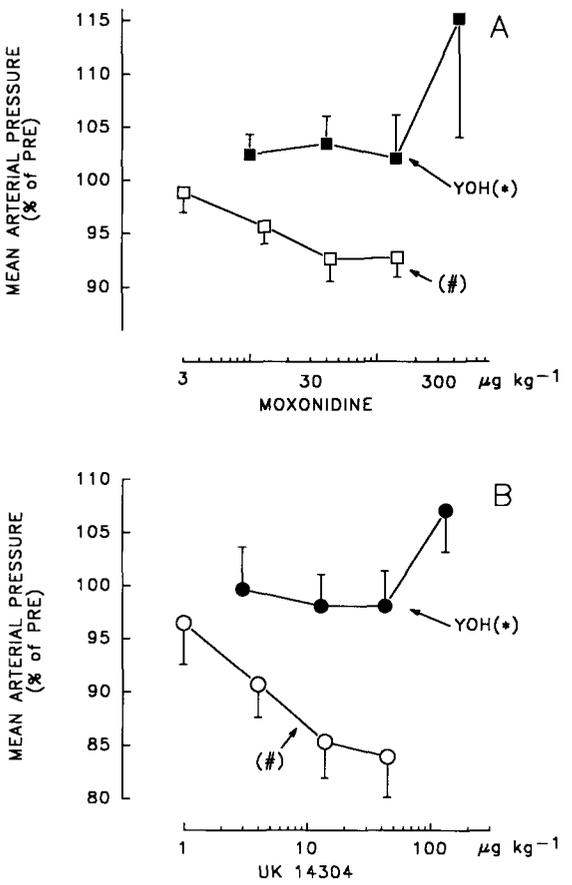

Fig. 7. Effect of (A) moxonidine and (B) UK 14304 on mean arterial pressure in rabbits pretreated *i.c.* with saline (empty symbols) or yohimbine (filled symbols; YOH). Protocol illustrated in Fig. 6. Mean arterial pressure was read about 10 min after injection of the respective dose of agonist (at next measurement point) and is expressed as a percentage of PRE (ordinates). Pretreatment dose of saline was 25 μl kg^{-1}. Dose of yohimbine was 1.5 μg kg^{-1}. Injected doses (i.v.) of moxonidine were 3, 10, 30 and 100 μg kg^{-1} in saline-pretreated and 10, 30, 100 and 300 μg kg^{-1} in yohimbine-pretreated rabbits. Injected doses (i.v.) of UK 14304 were 1, 3, 10 and 30 μg kg^{-1} in saline-pretreated and 3, 10, 30 and 90 μg kg^{-1} in yohimbine-pretreated rabbits. Abscissae show *cumulative* doses. Means ± S.E.M., $n = 6$–9. $^{\#}$ Significant difference ($P < 0.05$) from saline-pretreated rabbits given four injections of saline instead of agonist. * Significant difference ($P < 0.05$) from agonist effect in saline-pretreated rabbits.

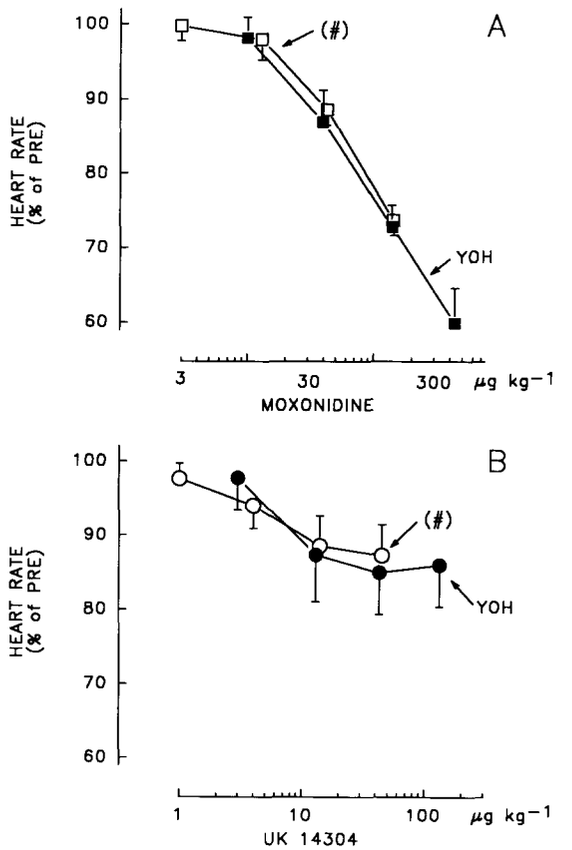

Fig. 8. Effect of (A) moxonidine and (B) UK 14304 on heart rate in rabbits pretreated *i.c.* with saline (empty symbols) or yohimbine (filled symbols; YOH). Heart rate was read about 10 min after injection of the respective dose of agonist (at next measurement point) and is expressed as a percentage of PRE (ordinates). See legend to Fig. 7 for details.

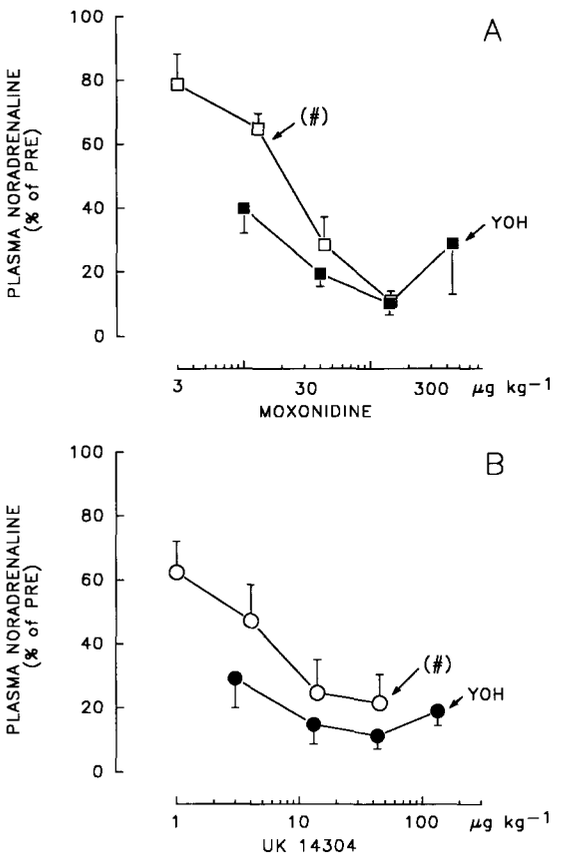

Fig. 9. Effect of (A) moxonidine and (B) UK 14304 on the plasma noradrenaline concentration in rabbits pretreated *i.c.* with saline (empty symbols) or yohimbine (filled symbols; YOH). Plasma for noradrenaline determination was obtained about 10 min after injection of the respective dose of agonist (at next measurement point) and the level is expressed as a percentage of PRE (ordinates). See legend to Fig. 7 for details.

the heart rate (Fig. 8B) and plasma noradrenaline (Fig. 9B) decreases produced by UK 14304.

4. Discussion

Moxonidine has previously been suggested to lower sympathetic outflow from the central nervous system (rat, rabbit: Armah et al., 1988; Schlicker et al., 1990; Haxhiu et al., 1992; cat: Ramage and Wilkinson, 1989; reviewed by Chrisp and Faulds, 1992; Molderings et al., 1993). In anaesthetized cats, the decrease of sympathetic outflow was shown directly by recording from peripheral sympathetic nerves (Ramage and Wilkinson, 1989). Our results for the first time demonstrate the central sympathoinhibition directly in conscious animals. The mechanism originally suggested was activation of central α_2-adrenoceptors (Armah et al., 1988; Ramage and Wilkinson, 1989; Schlicker et al., 1990). More recently, a primary action of moxonidine at imidazoline I_1 receptors in the RVLM has been proposed (Ernsberger et al., 1992,1993; Molderings et al., 1993; Haxhiu et al., 1994).

Which alternative holds? Following the design of a recent study on rilmenidine (Urban et al., 1994), we compared effects of moxonidine with those of the α_2-adrenoceptor agonist UK 14304, which has very little affinity for I_1 binding sites, and studied the antagonism against both of yohimbine, also highly selective for α_2-adrenoceptors as compared to I_1 sites (see Introduction).

If UK 14304 acted through α_2-adrenoceptors and moxonidine through I_1 receptors, then the patterns of their effects might be expected to differ. This was not found: an initial transient increase in mean arterial pressure followed by prolonged hypotension, bradycardia, inhibition of renal sympathetic firing and a decrease in the arterial plasma noradrenaline concentration were obtained for both compounds. The maximal hypotension was smaller for moxonidine than for UK 14304 in both series of experiments (i.v. and i.c. pretreatment with saline; Fig. 2 and Fig. 7), possibly due to some activation of vascular smooth muscle α_1-adrenoceptors by moxonidine (Armah, 1988) but not UK 14304 (Van Meel et al., 1981). Conversely, the bradycardia was more pronounced after moxonidine than after UK 14304, again in both series (Fig. 3 and Fig. 8), perhaps due to a greater baroreflex cardioaccelerator component in the case of UK 14304 with its greater hypotensive effect.

If UK 14304 acted through α_2- and moxonidine through I_1 receptors, then *i.v.* yohimbine might be expected to antagonize the effects of UK 14304 to a greater extent than those of moxonidine. This was not found: both the low and the high dose of yohimbine antagonized the effects of moxonidine on the one hand, and of UK 14304 on the other, in a similar manner. The similarity holds true for the inhibitory effects shown in Figs. 2, 3, 4 and 5 as well as for the initial hypertension, indicating that the latter, as expected, was at least partly due to activation of vascular smooth muscle α_2-adrenoceptors. There might seem to be two exceptions to the rule of similarity. First, yohimbine shifted the dose-hypotension curve of UK 14304 to the right in an apparently parallel manner but suppressed the hypotension caused by moxonidine completely (Fig. 2). Second, the low dose of yohimbine shifted the moxonidine dose-renal sympathoinhibition curve less to the right (and non-significantly) than the curve of UK 14304 (Fig. 4). However, these apparent exceptions need not reflect the involvement of different receptors in the central sympathoinhibition. The complete suppression of the hypotension (Fig. 2) may be due to the inherently low hypotensive efficacy of

moxonidine and its α_1 vasoconstrictor component (see above). The greater shift by the low dose of yohimbine of the dose-renal sympathoinhibition curve of UK 14304 (Fig. 4) may be due to a dual mechanism: competition by yohimbine for the sympathoinhibitory receptors *and* activation of the baroreflex by the UK 14304-induced blood pressure fall that persisted after the low dose of yohimbine (Fig. 2); only the former but not the latter mechanism operated in the case of moxonidine.

Finally, if UK 14304 acted through α_2- and moxonidine through I_1 receptors, then *i.c.* yohimbine (at less than 0.3% of the lower i.v. dose) should antagonize (central) effects of UK 14304 more than of moxonidine. Again, however, this was not verified: yohimbine i.c. interacted with moxonidine as it did with UK 14304. The transient hypertensive effects persisted, demonstrating lack of peripheral α-adrenoceptor blockade by yohimbine i.c. The hypotension, in contrast, was abolished, in accord with the view that central actions are necessary (although perhaps not sufficient: Urban et al., 1995) for systemically given clonidine-like drugs to lower blood pressure. Unexpectedly, yohimbine i.c. attenuated neither the decrease in heart rate nor the fall in plasma noradrenaline caused by moxonidine and UK 14304. The bradycardia after clonidine-like drugs has been attributed to activation of central α_2-adrenoceptors (Häusler, 1982; Kobinger, 1986; Kobinger and Pichler, 1990), although lack of, or only minimal, blockade by i.c. α_2-adrenoceptor antagonists has been reported previously (Brown and Harland, 1984; Tibiriça et al., 1991; Mayorov et al., 1993). The persistence of the plasma noradrenaline decrease (Fig. 9) stands in contrast to the blockade of the blood pressure decrease (Fig. 7), an apparent paradox previously observed in anaesthetized rats in which idazoxan i.c. almost abolished the hypotensive effect of clonidine but did not affect the plasma noradrenaline fall (Brown and Harland, 1984). The reason for the 2-fold absence of an expected antagonism probably is the limited distribution of yohimbine given i.c. Yohimbine i.c. did reach the RVLM, the main area where clonidine-like drugs lower blood pressure (see Introduction). However, it may not have reached at sufficient concentration brain regions where moxonidine and UK 14304 produced bradycardia (see Philippu, 1988; Molderings et al., 1993; Dampney, 1994) and inhibition of non-vasomotor sympathetic outflow. Moreover, there is evidence indicating that clonidine-like drugs owe part of their bradycardic effect to activation of *peripheral* cardiac presynaptic α_2-autoreceptors (De Jonge et al., 1981; Urban et al., 1995). Both moxonidine and UK 14304, given i.v. at the doses used in the present study, reduced the plasma noradrenaline level in pithed rabbits with electrically stimulated sympathetic outflow, demonstrating the role of peripheral α_2-autoreceptor activation in this response (Urban et al., 1995). Whatever the reason for the 2-fold lack of antagonism, it is remarkable how perfectly moxonidine and UK 14304 agreed.

One may still ask why the reduction of sympathetic tone remaining after yohimbine i.c., demonstrated by the unchanged plasma noradrenaline fall (Fig. 9) and probably at least partly due to peripheral presynaptic inhibition, was not accompanied by any hypotension (Fig. 7). The answer is that once the central hypotensive effect of clonidine-like drugs is occluded, the remaining presynaptic inhibition of vascular sympathetic transmitter release is balanced by activation of vascular smooth muscle α-adrenoceptors (Urban et al., 1995).

To summarize, the presumed I_1 site ligand moxonidine and the highly α_2-adrenoceptor selective agonist UK 14304 were closely similar in, first, their effects on mean arterial pressure, heart rate, renal sympathetic nerve activity and the plasma noradrenaline concentration when given alone; second, their interaction on these parameters with the highly α_2-selective antagonist yohimbine given i.v.; and third, their interaction with yohimbine given i.c. The results resemble and extend those of a study comparing the presumed I_1 site ligand rilmenidine with UK 14304 (Urban et al., 1994). The conclusion is analogous: α_2-adrenoceptors are involved in the central sympathoinhibitory effect of moxonidine whereas, from these experiments, there is no indication for participation of imidazoline I_1 receptors. The same conclusion has also been drawn in a recent study on clonidine (Hieble and Kolpak, 1993). We cannot exclude the possibility that clonidine-like drugs primarily act at I_1 receptors and that the α_2-adrenoceptors are a later necessary link in the pathway leading to central sympathoinhibition (Sannajust and Head, 1994). However, the virtual identity of the effects of rilmenidine (Urban et al., 1994) and moxonidine with those of UK 14304, together with the identical antagonism patterns, speak in favour of α_2-adrenoceptors as the *primary* sites of action. Moxonidine possesses high selectivity for I_1 in comparison with α_2 binding sites in membranes prepared from the bovine RVLM (see Introduction; Ernsberger et al., 1992,1993). Interestingly, recent studies indicate that moxonidine possesses very little affinity for the imidazoline binding sites in the ventrolateral medulla of rabbit and man (Bricca et al., 1993,1994).

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References

Armah, B.I., 1988, Unique presynaptic α_2-receptor selectivity and specificity of the antihypertensive agent moxonidine, Arzneim.-Forsch./Drug Res. 38, 1435.

Armah, B.I., E. Hofferber and W. Stenzel, 1988, General pharmacology of the novel centrally acting antihypertensive agent moxonidine, Arzneim.-Forsch./Drug Res. 38, 1426.

Bousquet, P., J. Feldman, R. Bloch and J. Schwartz, 1981, The nucleus reticularis lateralis: a region highly sensitive to clonidine, Eur. J. Pharmacol. 69, 389.

Bousquet, P., G. Bricca, M. Dontenwill, J. Feldman, H. Greney, A. Belcourt, J. Stutzmann and E. Tibiriça, 1992, From the α_2-adrenoceptors to the imidazoline preferring receptors, Fundam. Clin. Pharmacol. 6 (Suppl. 1), 15s.

Bricca, G., M. Dontenwill, A. Molines, J. Feldman, A. Belcourt and P. Bousquet, 1989, The imidazoline preferring receptor: binding studies in bovine, rat and human brainstem, Eur. J. Pharmacol. 162, 1.

Bricca, G., J. Zhang, H. Greney, M. Dontenwill, J. Stutzmann, A. Belcourt and P. Bousquet, 1993, Relevance of the use of [^{3}H]-clonidine to identify imidazoline receptors in the rabbit brainstem, Br. J. Pharmacol. 110, 1537.

Bricca, G., H. Greney, J. Zhang, M. Dontenwill, J. Stutzmann, A. Belcourt and P. Bousquet, 1994, Human brain imidazoline receptors: further characterization with [^{3}H]clonidine, Eur. J. Pharmacol. 266, 25.

Brown, M.J. and D. Harland, 1984, Evidence for a peripheral component in the sympatholytic effect of clonidine in rats, Br. J. Pharmacol. 83, 657.

Chrisp, P. and D. Faulds, 1992, Moxonidine, Drugs 44, 993.

Dampney, R.A.L., 1994, Functional organization of central pathways regulating the cardiovascular system, Physiol. Rev. 74, 323.

De Jonge, A., P.B.M.W.M. Timmermans and P.A. Van Zwieten, 1981, Participation of cardiac presynaptic α_2-adrenoceptors in the bradycardiac effects of clonidine and analogues, Naunyn-Schmied. Arch. Pharmacol. 317, 8.

Ernsberger, P., M.P. Meeley, J.J. Mann and D.J. Reis, 1987, Clonidine binds to imidazole binding sites as well as α_2-adrenoceptors in the ventrolateral medulla, Eur. J. Pharmacol. 134, 1.

Ernsberger, P.R., K.L. Westbrooks, M.O. Christen and S.G. Schäfer, 1992, A second generation of centrally acting antihypertensive agents act on putative I_1-imidazoline receptors, J. Cardiovasc. Pharmacol. 20 (Suppl. 4), S1.

Ernsberger, P., T.H. Damon, L.M. Graff, S.G. Schäfer and M.O. Christen, 1993, Moxonidine, a centrally acting antihypertensive agent, is a selective ligand for I_1-imidazoline sites, J. Pharmacol. Exp. Ther. 264, 172.

Häusler, G., 1982, Central α-adrenoceptors involved in cardiovascular regulation, J. Cardiovasc. Pharmacol. 4 (Suppl. 1), S72.

Haxhiu, M.A., I. Dreshaj, B. Erokwu, S.G. Schäfer, M.O. Christen and P.R. Ernsberger, 1992, Vasodepression elicited in hypertensive rats by the selective I_1-imidazoline agonist moxonidine administered into the rostral ventrolateral medulla, J. Cardiovasc. Pharmacol. 20 (Suppl. 4), S11.

Haxhiu, M.A., I. Dreshaj, S.G. Schäfer and P. Ernsberger, 1994, Selective antihypertensive action of moxonidine is mediated mainly by I_1-imidazoline receptors in the rostral ventrolateral medulla, J. Cardiovasc. Pharmacol. 24 (Suppl. 1), S1.

Head, G.A., P.I. Korner, S.L. Lewis and E. Badoer, 1983, Contribution of noradrenergic and serotonergic neurons to the circulatory effects of centrally acting clonidine and α-methyldopa in rabbits, J. Cardiovasc. Pharmacol. 5, 945.

Hieble, J.P. and D.C. Kolpak, 1993, Mediation of the hypotensive action of systemic clonidine in the rat by α_2-adrenoceptors, Br. J. Pharmacol. 110, 1635.

Kobinger, W., 1986, Drugs as tools in research on adrenoceptors, Naunyn-Schmied. Arch. Pharmacol. 332, 113.

Kobinger, W. and L. Pichler, 1990, Centrally acting drugs (clonidine, methyldopa, guanfacine), in: Handbook of Experimental Pharmacology, Pharmacology of Antihypertensive Therapeutics, Vol. 93, eds. D. Ganten and P.J. Mulrow (Springer-Verlag, Berlin) p. 227.

Mayorov, D., M. Chernobelski and O. Medvedev, 1993, Sympathoinhibitory action of rilmenidine in conscious sinoaortically denervated rats, J. Cardiovasc. Pharmacol. 22, 314.

Molderings, G.J., M. Göthert, O. Christen and S.G. Schäfer, 1993, Imidazolrezeptoren und Blutdruckregulation, Dtsch. Med. Wochenschr. 118, 953.

Philippu, A., 1988, Regulation of blood pressure by central neurotransmitters and neuropeptides, Rev. Physiol. Biochem. Pharmacol. 111, 1.

Ramage, A.G. and S.J. Wilkinson, 1989, Evidence that different regional sympathetic outflows vary in their sensitivity to the sympathoinhibitory actions of putative 5-HT_{1A} and α_2-adrenoceptor agonists in anaesthetized cats, Br. J. Pharmacol. 98, 1157.

Sannajust, F. and G.A. Head, 1994, Rilmenidine-induced hypotension in conscious rabbits involves imidazoline-preferring receptors, J. Cardiovasc. Pharmacol. 23, 42.

Schlicker, E., B.I. Armah and M. Göthert, 1990, Central presynaptic α_2-autoreceptors are involved in the blood pressure-lowering effect of moxonidine, J. Cardiovasc. Pharmacol. 16, 15.

Szabo, B., L. Hedler and K. Starke, 1989, Peripheral presynaptic and central effects of clonidine, yohimbine and rauwolscine on the sympathetic nervous system in rabbits, Naunyn-Schmied. Arch. Pharmacol. 340, 648.

Szabo, B. and A. Schultheiss, 1990, Desipramine inhibits sympathetic nerve activity in the rabbit, Naunyn-Schmied. Arch. Pharmacol. 342, 469.

Szabo, B., R. Urban and K. Starke, 1993, Sympathoinhibition by rilmenidine in conscious rabbits: involvement of α_2-adrenoceptors, Naunyn-Schmied. Arch. Pharmacol. 348, 593.

Tibiriça, E., J. Feldman, C. Mermet, F. Gonon and P. Bousquet, 1991, An imidazoline-specific mechanism for the hypotensive effect of clonidine: a study with yohimbine and idazoxan, J. Pharmacol. Exp. Ther. 256, 606.

Urban, R., B. Szabo and K. Starke, 1994, Is the sympathoinhibitory effect of rilmenidine mediated by alpha-2 adrenoceptors or imidazoline receptors?, J. Pharmacol. Exp. Ther. 270, 572.

Urban, R., B. Szabo and K. Starke, 1995, Involvement of peripheral presynaptic inhibition in the reduction of sympathetic tone by moxonidine, rilmenidine and UK 14304, Eur. J. Pharmacol. 282, 29.

Van Meel, J.C.A., A. De Jonge, P.B.M.W.M. Timmermans and P.A. Van Zwieten, 1981, Selectivity of some alpha adrenoceptor agonists for peripheral alpha-1 and alpha-2 adrenoceptors in the normotensive rat, J. Pharmacol. Exp. Ther. 219, 760.

Van Zwieten, P.A., 1988, Pharmacology of the alpha$_2$-adrenoceptor agonist rilmenidine, Am. J. Cardiol. 61, 6D.

Verbeuren, T.J., A.T.D. Xuan, E. Koenig-Bérard and P. Vitou, 1990, Rilmenidine, Cardiovasc. Drug. Rev. 8, 56.