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Effects of imidazoline antihypertensive drugs on sympathetic tone and noradrenaline release in the prefrontal cortex

*,[1]Bela Szabo, [1]Thomas Fritz & [1,2]Krzysztof Wedzony

[1]Institute of Experimental and Clinical Pharmacology and Toxicology, Albert Ludwigs University, Hermann-Herder-Strasse 5, D-79104 Freiburg i. Br., Germany and [2]Institute of Pharmacology, Polish Academy of Sciences, 31-343 Krakow, 12 Smetna Street, Poland

1 The aim of the present study was to compare the effects of the centrally acting antihypertensive drugs rilmenidine, moxonidine, clonidine and guanabenz on sympathetic tone with their effects on noradrenaline release in the cerebral cortex. In particular, the hypothesis was tested that rilmenidine and moxonidine, due to their high affinity for sympatho-inhibitory imidazoline I_1 receptors and low affinity for α_2-adrenoceptors, lower sympathetic tone without causing an α_2-adrenoceptor-mediated inhibition of cerebrocortical noradrenaline release.

2 In rats anaesthetized with urethane, blood pressure and heart rate were measured and the concentration of noradrenaline in arterial blood plasma was determined. The release of noradrenaline in the medial prefrontal cortex was estimated by microdialysis. Intravenous administration of rilmenidine (30, 100, 300 and 1000 μg kg^{-1}), moxonidine (10, 30, 100 and 300 μg kg^{-1}), clonidine (1, 3, 10 and 30 μg kg^{-1}) and guanabenz (1, 3, 10 and 30 μg kg^{-1}) led to dose-dependent hypotension and bradycardia; the plasma noradrenaline concentration also decreased. After the two highest doses, all four drugs lowered noradrenaline release in the prefrontal cortex. At doses eliciting equal hypotensive and sympatho-inhibitory responses, rilmenidine and moxonidine inhibited cerebral cortical noradrenaline release at least as much as clonidine and guanabenz.

3 The results show that rilmenidine and moxonidine lower cerebrocortical noradrenaline release at doses similar to those which cause sympatho-inhibition. This effect was probably due to an α_2-adrenoceptor-mediated inhibition of the firing of locus coeruleus neurons and, in addition, to presynaptic inhibition of noradrenaline release at the level of the axon terminals in the cortex. The results argue against the hypothesis that rilmenidine and moxonidine, due to their selectivity for sympatho-inhibitory I_1 imidazoline receptors, do not suppress noradrenergic neurons in the central nervous system.

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Keywords: Clonidine; rilmenidine; moxonidine; blood pressure; heart rate; plasma noradrenaline; cerebrocortical noradrenaline release; microdialysis; α_2-adrenoceptor; imidazoline receptor

Abbreviations: PRE, average of initial values (before drug application); RVLM, rostral ventrolateral medulla oblongata

Introduction

Clonidine, rilmenidine and moxonidine are centrally acting antihypertensive drugs. It is generally accepted that these drugs lower sympathetic outflow to the tissues, but the mechanism of the sympatho-inhibition is debated.

The initial hypothesis, originally developed for clonidine, suggests that activation of α_2-adrenoceptors in the medulla oblongata, most probably in the rostral ventrolateral medulla oblongata (RVLM), is responsible for the sympatho-inhibition (for review see Kobinger & Pichler, 1990; Guyenet, 1997; Eglen *et al*., 1998). A more recent hypothesis (which we call 'imidazoline hypothesis') assumes that sympatho-inhibitory imidazoline I_1 receptors in the RVLM are the primary targets of these drugs (for review see Reis, 1996; Ernsberger & Haxhiu, 1997; Bousquet & Feldman, 1999). In radioligand binding studies, clonidine, rilmenidine and moxonidine possess affinity for both α_2-adrenoceptors and imidazoline I_1 binding sites (Ernsberger *et al*., 1993; Molderings *et al*., 1993; Bricca *et al*., 1993; 1994; Piletz *et al*., 1996). According to Ernsberger *et al*. (1993), rilmenidine and moxonidine possess about 30 fold higher affinity for I_1 binding sites than for α_2-adrenoceptors.

An important component of the imidazoline hypothesis deals with the relationship between the sympatho-inhibitory effect and the side effects of the imidazoline drugs. It is suggested that clonidine, due to its comparable affinity for I_1 imidazoline receptors and α_2-adrenoceptors, simultaneously lowers sympathetic tone and elicits the α_2-adrenoceptor-mediated side effects sedation and dry mouth. In contrast, rilmenidine and moxonidine, due to their selectivity for the sympatho-inhibitory I_1 imidazoline receptor, should cause less α_2-adrenoceptor-mediated side effects (Ernsberger *et al*., 1993; Reis, 1996; Bousquet & Feldman, 1999). The sedative effect of clonidine-like drugs is attributed to inhibition of the firing of locus coeruleus neurons (De Sarro *et al*., 1987; Correa-Sales *et al*., 1992). The mechanism of the inhibition of saliva secretion is less clear: primary actions on the brain stem, on presynaptic terminals of postganglionic cholinergic

*Author for correspondence; E-mail: szabo@uni-freiburg.de

neurons in the salivary glands and on secretory gland cells are all possible (Green *et al*., 1979; Lung, 1994; Izumi *et al*., 1995).

The aim of the present study was to compare the sympatho-inhibitory efffects of clonidine-like drugs with their effects on central noradrenergic neurons. Specifically, the assumption of the imidazoline hypothesis was tested that rilmenidine and moxonidine, due to their high affinity for sympatho-inhibitory imidazoline I_1 receptors and low affinity for α_2-adrenoceptors, lower only sympathetic tone without causing an α_2-adrenoceptor-mediated inhibition of cerebrocortical noradrenaline release. Blood pressure, heart rate and the plasma noradrenaline concentration were determined as measures of sympathetic tone in anaesthetized rats. The activity of central noradrenergic neurons was estimated by measuring the extracellular noradrenaline concentration in the medial prefrontal cortex by means of microdialysis. The medial prefrontal cortex receives its noradrenergic innervation exclusively from the locus coeruleus (Aston-Jones *et al*., 1995); accordingly, the extracellular noradrenaline concentration in this region, measured by microdialysis, reflects the activity of locus coeruleus neurons (Van Gaalen *et al*., 1997; Berridge & Abercrombie, 1999). In addition to rilmenidine and moxonidine, clonidine and guanabenz were also studied for comparison. Guanabenz is a centrally acting antihypertensive drug, which proved to be selective for α_2-adrenoceptors in radioligand binding experiments (*vs* I_1 imidazoline binding sites; Ernsberger *et al*., 1993; Piletz *et al*., 1996; but see also Hamilton *et al*., 1991).

Methods

Sixty-one male Wistar rats weighing 250–300 g were used; they were obtained from Charles River (Sulzfeld, Germany). The experiments conformed to the rules of the German law regulating the use of animals in biomedical research (Tierschutzgesetz) and were approved by a local ethical committee.

Preparation

To produce anaesthesia, 0.2 g kg^{-1} of urethane was injected intraperitoneally; additional 0.2 g kg^{-1} doses were given i.v. every 2 h. Surgery started 50 min after initiation of anaesthesia. The animals were placed on a heated pad to keep rectal temperature at 37°C. The head was then fixed in a David Kopf stereotaxic frame; the incisor bar was set 10 mm below the interaural line. A hole (diameter, 2.5 mm) was made in the skull and the dura mater 3.1 mm rostrally from the bregma and 0.5 mm laterally from the midline, on the right side. The animal was then removed from the stereotaxic frame.

A tracheotomy was performed and the trachea was cannulated with polyethylene tubing (outer diameter, 2.1 mm; internal diameter, 1.6 mm). The left femoral artery and vein were cannulated with polyethylene tubing (outer diameter, 1 mm; internal diameter, 0.5 mm). One ml of saline containing heparin (10 U ml^{-1}) was injected through the arterial catheter to prevent blood clotting. Treatment with heparin was essential to keep the arterial catheter patent. On the other hand, the hole in the skull and dura mater had to be prepared before administration of heparin to avoid strong bleeding from the wound.

The head of the animal was again positioned in the stereotaxic frame. A microdialysis probe was inserted into the medial prefrontal cortex (see Figure 1A); the tip of the probe lay 3.1 mm rostrally from bregma, 0.5 mm laterally from midline and 5 mm ventrally from the surface of the brain (compare with Paxinos & Watson, 1982). Artificial ventilation with room air, at a rate of 50 min^{-1}, was started.

Immediately after the start of ventilation, pancuronium (1 mg kg^{-1}, i.v.) was injected to block neuromuscular transmission; 0.5 mg kg^{-1} of pancuronium were given additionally every 2 h.

At the end of the experiments, rats were killed with an overdose of urethane. The brain was removed and fixed in physiological saline containing 3.3% paraformaldehyde (v v^{-1}). Coronal brain slices (50 μm thick) were cut with a vibratome and the correct localization of the microdialysis probe in the medial prefrontal cortex was verified under a microscope (see Figure 1A).

Recording of blood pressure and heart rate

Femoral arterial blood pressure was measured with a Baxter/Uniflow transducer coupled to a bridge amplifier (Hugo Sachs Elektronik, Hugstetten, Germany). The heart rate was calculated from the pulsatile blood pressure signal by an integrator (Hugo Sachs Elektronik, Hugstetten, Germany).

Determination of the plasma concentration of noradrenaline

The method was similar to that used for determination of catecholamines in rabbit blood plasma (Szabo & Schultheiss, 1990). Blood (250 μl) was sampled from the femoral arterial catheter and immediately centrifuged (12,000 × *g*; 3 min; 0°C). One hundred μl of the plasma was frozen (−80°C) in tubes containing 5 μl of Na_2SO_3 (12.5%) and 5 μl of Na_2EDTA (10%). Erythrocytes were resuspended by adding 100 μl saline and reinjected after the next blood sampling.

On the day of the analysis, the frozen sample (110 μl) was thawed. After centrifugation (2500 × *g*, 10 min, 4°C), 100 μl of the supernatant was filled into a filtration unit (Centrisart-C4; Sartorius, Göttingen, Germany) and mixed for 10 min with 25 μl of Na_2SO_3 (1%), 25 μl of Na_2EDTA (1.25%), 100 μl of TRIS-HCl (2 mol l^{-1}; pH, 8.3) and 10 mg of Al_2O_3. The supernatant was removed by centrifugation (1000 × *g*, 2 min, 4°C), and the alumina was washed twice with 100 μl of distilled water. Noradrenaline was eluted from the alumina with 2 × 75 μl of $HClO_4$ (0.1 mol l^{-1}); each washing and elution step was terminated by centrifugation (1000 × *g*, 2 min, 4°C).

Noradrenaline in 100 μl $HClO_4$ eluate was quantified with an HPLC system consisting of a Waters 515 pump (Waters, Eschborn, Germany), a Waters 717plus sample injector and an Intro electrochemical detector (Antec Leyden, Leiden, The Netherlands). The chromatograms were evaluated by the software Millenium from Waters. The stationary phase was a Prontosil column (125 × 4 mm, 3 μm particle size; Bischoff, Leonberg, Germany); the column temperature was kept at 38°C by a Waters column thermostat. The mobile

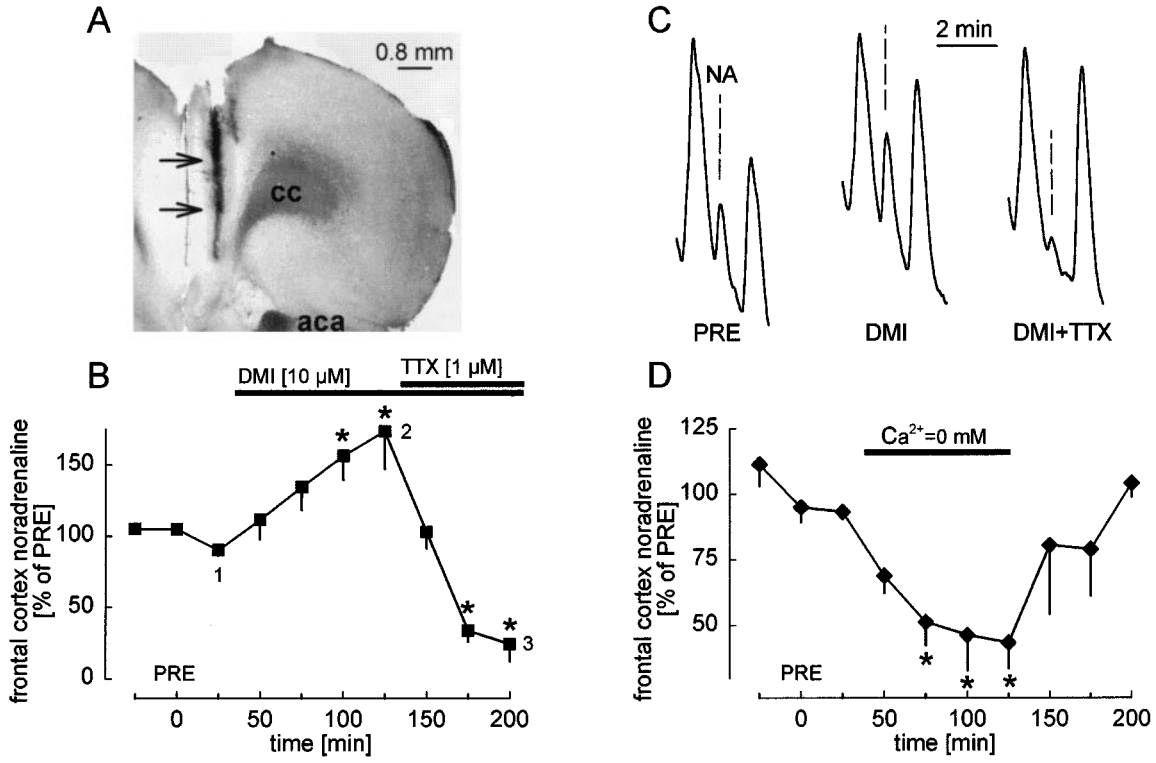

Figure 1 Characterization of the microdialysis method. (A) Coronal brain section showing localization of the microdialysis probe (arrows) in the medial prefrontal cortex. The 50 μm slice was obtained approximately 3.1 mm rostrally from the bregma. Abbreviations: cc, corpus callosum; aca, commissura anterior. (B) Effects of local application of desipramine (DMI, 10 μM) and tetrodotoxin (TTX, 1 μM) with the microdialysis fluid on the extracellular noradrenaline concentration in the medial prefrontal cortex. (C) Typical chromatograms of microdialysis samples. The samples were obtained before (PRE) and during local application of DMI (10 μM) and DMI (10 μM) plus TTX (1 μM) (at time points 1–3 shown in B). The dashed line indicates the retention time of noradrenaline in the standard chromatography sample (4.7 min). (D) Effect of perfusion of artificial cerebrospinal fluid with zero calcium concentration. Means ± s.e.mean from seven (DMI + TTX) and five (Ca^{2+} = 0 mM) experiments. Significant differences from PRE: $^*P<0.05$.

phase had a flow rate of 0.8–1 ml min^{-1} and consisted of (mmol l^{-1}): sodium acetate 87, citric acid 31, Na_2EDTA 1.3 and sodium octylsulphate 3.1; the concentration of methanol was 7.7% (v v^{-1}); the pH was 5.3 (adjusted with NaOH). The recovery of 200 pg noradrenaline added to 100 μl plasma was 72%.

Determination of the noradrenaline concentration in the prefrontal cortex

A microdialysis probe with a 3 mm-long active tip, prepared of polyether sulphone (PES) with a cut-off value of 35,000 Dalton, was used (MAB 2.14.3; Metalant, Stockholm, Sweden). The probe was perfused at a rate of 1.2 μl min^{-1} with artificial cerebrospinal fluid of the following composition (mmol l^{-1}): NaCl 147, KCl 4, $CaCl_2$ 2.4, $MgCl_2$ 1 (pH 7.4, adjusted with NaOH). To facilitate measurement of noradrenaline, neuronal reuptake of noradrenaline was blocked by adding desipramine (final concentration, 10 μmol l^{-1}) to the artificial cerebrospinal fluid. Twenty-five min aliquots of the perfusate (30 μl) were collected in chilled tubes containing 5 μl of $HClO_4$ (0.1 mol l^{-1}); samples were then stored at −80°C. The noradrenaline content of 25 μl perfusate was determined with the same HPLC system which was used for the determination of the plasma noradrenaline concentration (see above).

Protocol and evaluation of experiments

The surgical preparation and positioning of the microdialysis probe lasted for about 60 min. A 150-min stabilization period followed, in order to reach constant cardiovascular and cortical noradrenaline release values; the end of this period was defined as t = 0 min.

In some experiments, the microdialysis probe was initially perfused with desipramine-free artificial cerebrospinal fluid, and desipramine and tetrodotoxin were later added to the fluid (between t = 27 and 200 min) in order to characterize the method (Figure 1). The effect of calcium removal was tested by perfusing calcium-free cerebrospinal fluid (Figure 1); since no calcium-chelating substance was added to the perfusion fluid, it cannot be expected that the concentration of calcium in the microdialysed extracellular space decreased to zero.

In the majority of experiments, drugs were administered intravenously. Thus, saline (1 ml kg^{-1}) or increasing doses of rilmenidine (30, 100, 300 and 1000 μg kg^{-1}), moxonidine (10, 30, 100 and 300 μg kg^{-1}), clonidine (1, 3, 10 and 30 μg kg^{-1}) or guanabenz (1, 3, 10 and 30 μg kg^{-1}) were injected i.v. at t = 27, 52, 77 and 102 min (immediately after blood samplings and dialysis sample collection periods; Figures 2–5).

Blood pressure and heart rate were read every 5 min from t = 0 to 125 min. Blood was sampled at t = 0, 25, 50, 75, 100 and 125 min for the determination of the plasma noradrena-

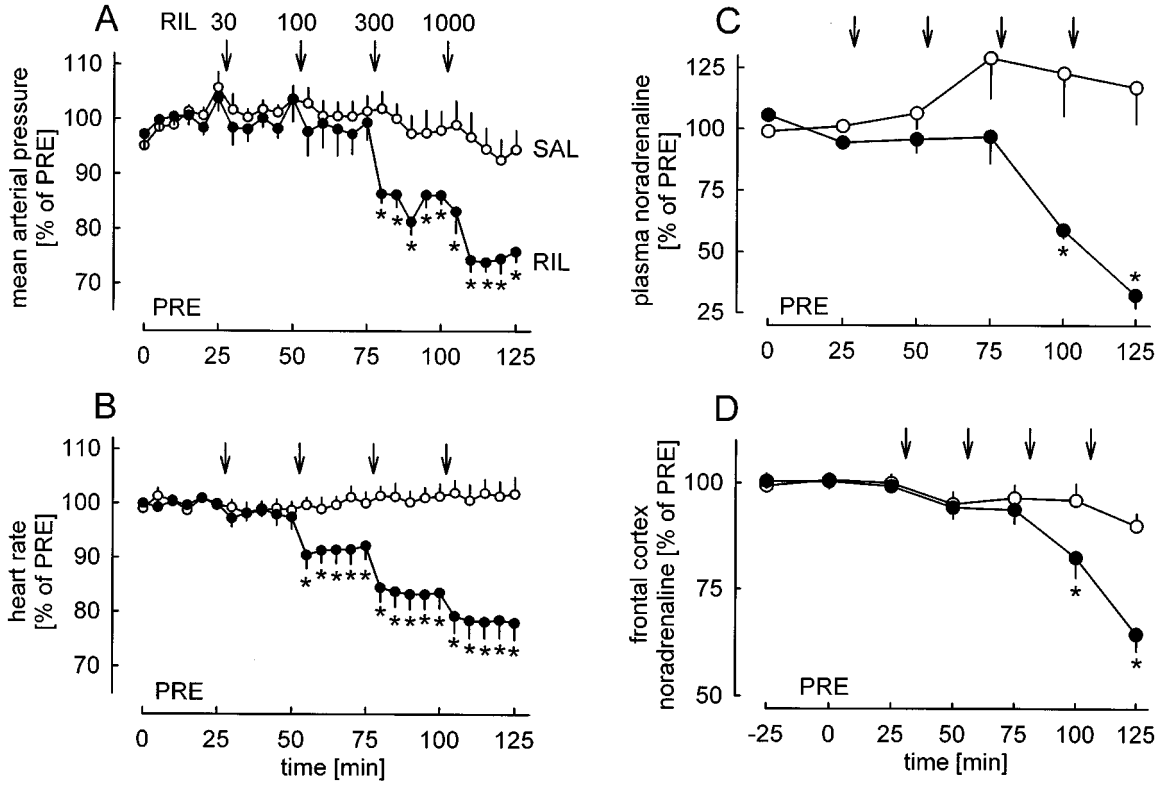

Figure 2 Effects of saline (SAL) and rilmenidine (RIL) on mean arterial pressure, heart rate, plasma noradrenaline concentration and extracellular noradrenaline concentration in the medial prefrontal cortex. SAL (1 ml kg^{-1}) or RIL (30, 100, 300 and 1000 μg kg^{-1}) were injected i.v. at t = 27, 52, 77 and 102 min as indicated by the arrows. Initial values before administration of saline or drugs were averaged to yield the PRE values and all values of an experiment were expressed as percentages of PRE. Means ± s.e.mean of 10 (SAL) and eight (IRL) experiments. Significant differences from SAL: $*P<0.05$.

line concentration. Cortical dialysis effluate was collected in 25-min samples from t = −50 to t = 125 min. Average baseline values (PRE values; Figures 2–5) were determined as follows. For blood pressure and heart rate, the six values read between t = 0 and 25 min were averaged to yield the PRE values. For plasma noradrenaline, the values of the blood samples obtained at t = 0 and 25 min were averaged to yield the PRE values. For cortical noradrenaline, values in the effluate collection periods which ended at t = −25, 0 and 25 min were averaged to obtain the PRE values. All values of an experiment were then expressed as percentages of PRE.

Statistics

Means ± s.e.mean of n experiments are given throughout. Since it is difficult to verify that criteria of parametric tests (Wallenstein *et al*., 1980) are fulfilled under our conditions, non-parametric tests were used (included in the statistical software SPSS for Windows v. 10.0; SPSS, Chicago, IL, U.S.A.). Differences between groups were evaluated with the one-tailed Mann-Whitney test. For correction of errors arising from multiple comparisons with the same control group, the simple sequentially rejective Bonferroni test was employed (Holm, 1979). Differences within groups were analysed with the Wilcoxon signed rank test. One experiment with rilmenidine was not included in the statistical analysis, because it contained several values in several parameters which fulfilled the criteria of outlying observations (Grubbs & Beck, 1972). $P<0.05$ was taken as the limit of statistical significance and only this level is indicated even if P was <0.01 or <0.001.

Drugs

Drugs were obtained from the following sources: clonidine HCl from Boehringer (Ingelheim, Germany), desipramine HCl and tetrodotoxin from Sigma (Deisenhofen, Germany), guanabenz acetate from Wyeth (Philadelphia, U.S.A.), heparin Na from Braun (Melsungen, Germany), moxonidine from Beiersdorf-Lilly (Hamburg, Germany), pancuronium Br from Organon Teknika (Eppenheim, Germany), rilmenidine dihydrogenphosphate from Servier (Courbevoie, France) and urethane from Serva (Heidelberg, Germany).

All drugs were dissolved in saline except tetrodotoxin (dissolved in buffer containing citric acid). Intravenous (i.v.) injections had a volume of 1 ml kg^{-1}. Doses refer to the salts.

Results

Characterization of the microdialysis method

Local administration of desipramine (10 μM) with the microdialysis fluid increased the noradrenaline content in the microdialysis samples by maximally 73% (Figure 1B,C). When, in addition to desipramine, tetrodotoxin (1 μM) was

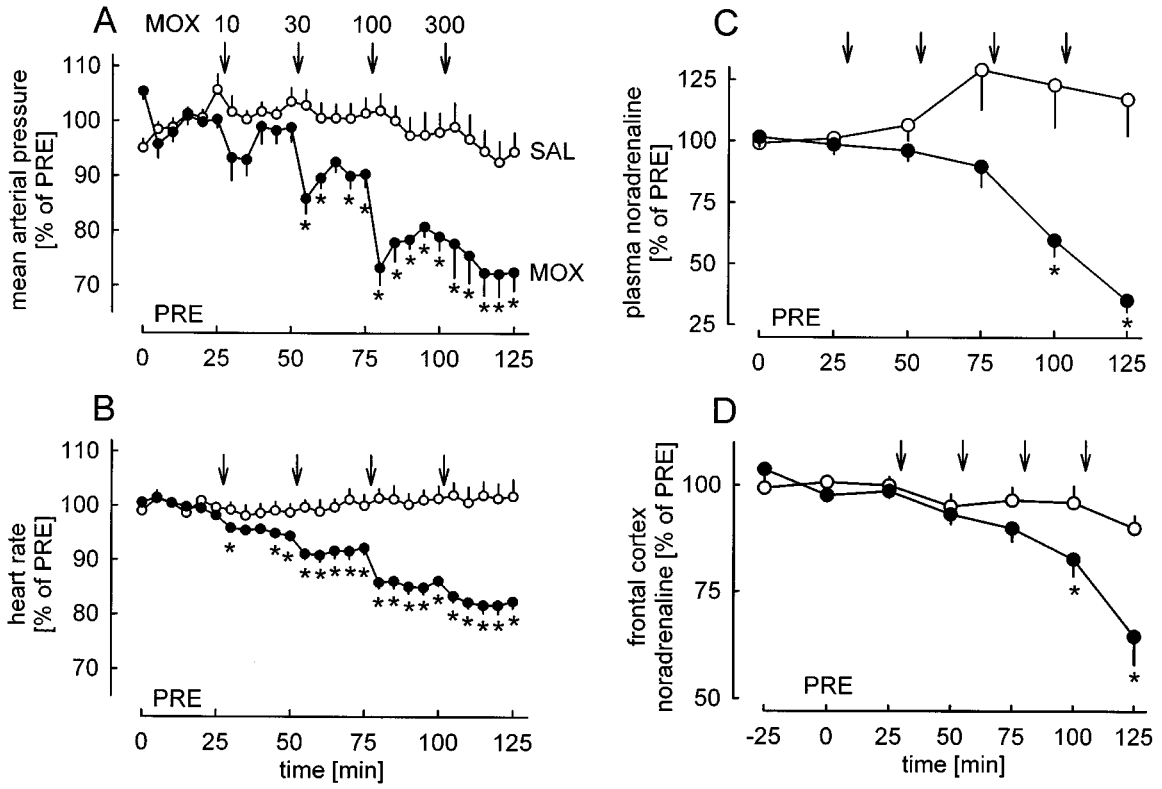

Figure 3 Effects of saline (SAL) and moxonidine (MOX) on mean arterial pressure, heart rate, plasma noradrenaline concentration and extracellular noradrenaline concentration in the medial prefrontal cortex. SAL (1 ml kg^{-1}) or MOX (10, 30, 100 and 300 $\mu g\ kg^{-1}$) were injected i.v. at t = 27, 52, 77 and 102 min as indicated by the arrows. Initial values before administration of saline or drugs were averaged to yield the PRE values and all values of an experiment were expressed as percentages of PRE. Means ± s.e.mean of 10 (SAL) and nine (MOX) experiments. Significant differences from SAL: $*P<0.05$.

administered locally, it greatly decreased the amount of noradrenaline in the microdialysis samples (Figure 1B,C). Perfusion of artificial cerebrospinal fluid with zero calcium ion concentration also significantly decreased the noradrenaline concentration in the dialysis samples (Figure 1D). The effects of desipramine, tetrodotoxin and calcium removal are similar to previous observations (Dalley & Stanford, 1995; Carter, 1997), and indicate that the noradrenaline appearing in the microdialysis fluid was released from noradrenergic axon terminals in an action potential- and calcium-dependent fashion. In further experiments, desipramine (10 μM) was always included in the perfusion fluid in order to facilitate measurement of noradrenaline.

Initial values, stability of parameters in control experiments

The initial baseline values (PRE values) were determined before administration of saline or hypotensive drugs. Mean arterial pressure and heart rate were 80 ± 2 mmHg and 450 ± 6 beats min^{-1} ($n = 49$), respectively, at this time point. The plasma concentration of noradrenaline during the initial period was 762 ± 49 pg ml^{-1} ($n = 44$). The noradrenaline content in the 25-min microdialysis samples was 34 ± 2 pg ($n = 45$) at the beginning of the experiments. For further evaluation, all values of an experiment were expressed as percentages of the initial PRE values.

In control experiments, saline (1 ml kg^{-1}) was injected i.v. four times. Blood pressure, heart rate, the plasma concentration of noradrenaline and the amount of noradrenaline in the microdialysis samples remained fairly constant until the end of these experiments (see the identical control curves in Figures 2–5; empty circles).

Effects of blood pressure lowering drugs

Rilmenidine (30, 100, 300 and 1000 $\mu g\ kg^{-1}$), moxonidine (10, 30, 100 and 300 $\mu g\ kg^{-1}$), clonidine (1, 3, 10 and 30 $\mu g\ kg^{-1}$) and guanabenz (1, 3, 10 and 30 $\mu g\ kg^{-1}$) were given i.v. in a cumulative fashion. These doses were selected with the aim to include doses which elicited minimal and maximal hypotensive responses. The maximal hypotensive response was a 25–30% decrease in mean arterial pressure, and this was evoked by all four hypotensive drugs. The pattern of effects of the four drugs was identical: blood pressure, heart rate, the plasma concentration of noradrenaline and cerebrocortical noradrenaline release decreased dose-dependently.

At the smallest dose (30 $\mu g\ kg^{-1}$), rilmenidine elicited no effects (Figure 2). The only significant effect of rilmenidine (100 $\mu g\ kg^{-1}$) was bradycardia. At the highest doses (300 and 1000 $\mu g\ kg^{-1}$), rilmenidine significantly depressed blood pressure, heart rate and the plasma noradrenaline concentration. Cortical noradrenaline release was also inhibited after the two highest doses of rilmenidine.

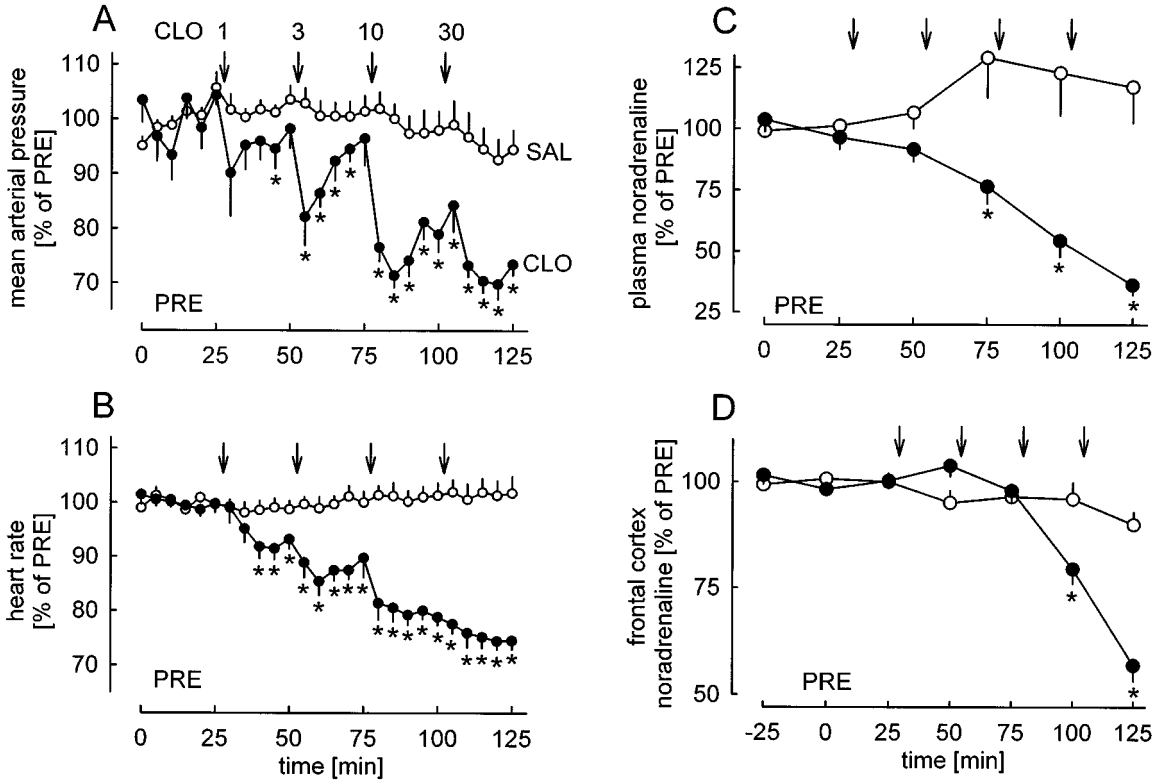

Figure 4 Effects of saline (SAL) and clonidine (CLO) on mean arterial pressure, heart rate, plasma noradrenaline concentration and extracellular noradrenaline concentration in the medial prefrontal cortex. SAL (1 ml kg^{-1}) or CLO (1, 3, 10 and 30 $\mu g\ kg^{-1}$) were injected i.v. at t = 27, 52, 77 and 102 min as indicated by the arrows. Initial values before administration of saline or drugs were averaged to yield the PRE values and all values of an experiment were expressed as percentages of PRE. Means ± s.e.mean of 10 (SAL) and eight (CLO) experiments. Significant differences from SAL: $^*P < 0.05$.

The lowest dose of moxonidine (10 $\mu g\ kg^{-1}$) caused only a small bradycardia (Figure 3). Moxonidine (30 $\mu g\ kg^{-1}$) decreased blood pressure and heart rate. The highest doses of moxonidine (100 and 300 $\mu g\ kg^{-1}$) simultaneously depressed blood pressure, heart rate, the plasma noradrenaline concentration and cortical noradrenaline release.

The smallest dose of clonidine (1 $\mu g\ kg^{-1}$) significantly lowered blood pressure and heart rate but did not change the plasma noradrenaline concentration and cortical noradrenaline release (Figure 4). Clonidine (3 $\mu g\ kg^{-1}$) significantly lowered blood pressure, heart rate and the plasma noradrenaline concentration. The highest doses of clonidine (10 and 30 $\mu g\ kg^{-1}$) significantly depressed all four parameters.

The smallest dose of guanabenz (1 $\mu g\ kg^{-1}$) elicited no significant effect (Figure 5). Guanabenz (3 $\mu g\ kg^{-1}$) significantly lowered blood pressure and heart rate. The highest doses of guanabenz (10 and 30 $\mu g\ kg^{-1}$) significantly depressed blood pressure, heart rate, the plasma noradrenaline concentration and cortical noradrenaline release.

Correlation between parameters

The decrease in blood pressure and plasma noradrenaline concentration was accompanied by a decrease in prefrontal cortex noradrenaline concentration in the case of all four hypotensive agents (Figure 6A,B). The graphic presentation allows comparison of the effects of the drugs on cortical noradrenaline release at doses which cause equal hypotension (Figure 6A) or equal sympatho-inhibition (Figure 6B). No major differences between the four drugs are apparent. Notably, at equi-hypotensive or equi-sympatho-inhibitory doses, rilmenidine and moxonidine lowered cortical noradrenaline concentration like clonidine. After the lower doses, rilmenidine and moxonidine even seemed to cause more depression of cortical noradrenaline release than clonidine.

Discussion

As expected from drugs classified as centrally acting antihypertensive agents, rilmenidine, moxonidine, clonidine and guanabenz lowered the sympathetic tone. This became manifest as a decrease in the plasma noradrenaline concentration. Consequently, blood pressure and heart rate also decreased.

In addition, all four drugs inhibited the release of noradrenaline in the medial prefrontal cortex. Two mechanisms are likely to be responsible for this inhibition. The more important mechanism probably was inhibition of firing of neurons in the locus coeruleus, which is the exclusive source of the noradrenergic input to the prefrontal cortex (Aston-Jones *et al*., 1995). It is known that clonidine (e.g., Svensson *et al*., 1975; Engberg & Eriksson, 1991; Berridge & Abercrombie, 1999) and rilmenidine (Dresse & Scuvée-Moreau, 1986) inhibit the firing of locus coeruleus neurons *in vivo*. Clonidine, rilmenidine and moxonidine lower the

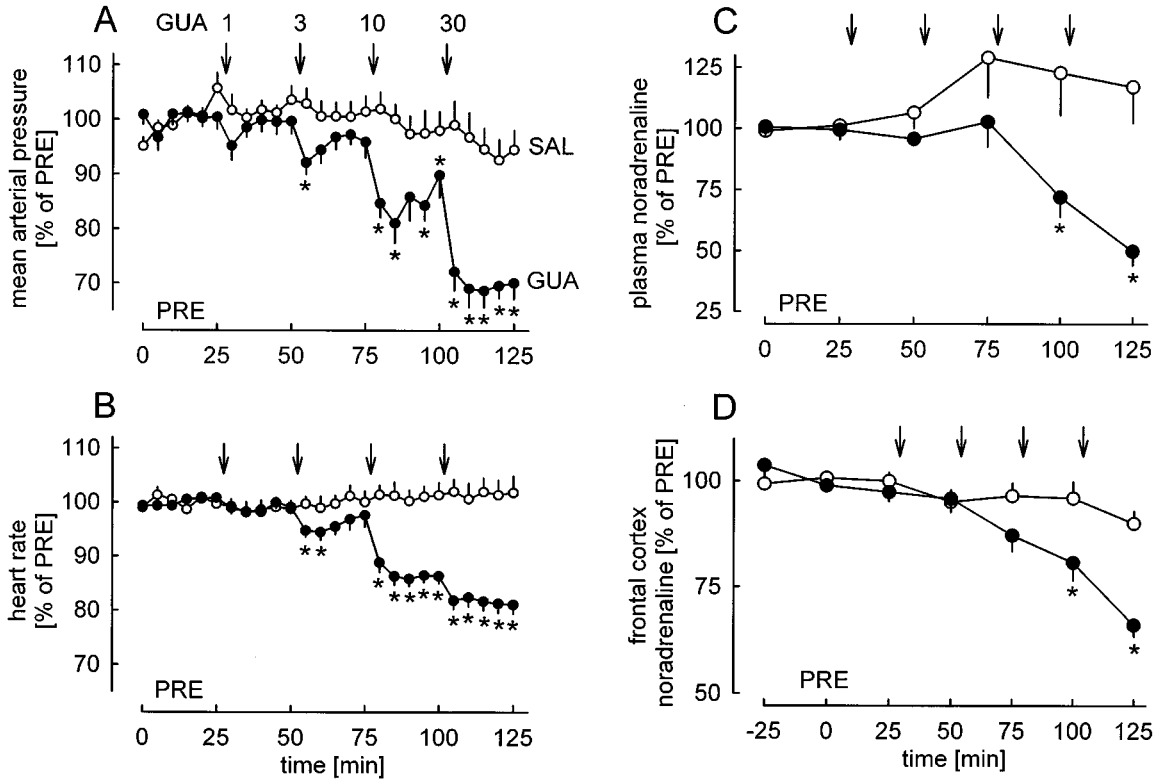

Figure 5 Effects of saline (SAL) and guanabenz (GUA) on mean arterial pressure, heart rate, plasma noradrenaline concentration and extracellular noradrenaline concentration in the medial prefrontal cortex. SAL (1 ml kg^{-1}) or GUA (1, 3, 10 and 30 μg kg^{-1}) were injected i.v. at t = 27, 52, 77 and 102 min as indicated by the arrows. Initial values before administration of saline or drugs were averaged to yield the PRE values and all values of an experiment were expressed as percentages of PRE. Means ± s.e.mean of 10 (SAL) and 13 (GUA) experiments. Significant differences from SAL: $^*P < 0.05$.

firing rate of locus coeruleus neurons also in brain slices (e.g., Williams *et al*., 1985; Szabo *et al*., 1996). It is noteworthy that the direct inhibitory effects of the clonidine-like drugs on locus coeruleus neurons is probably weakened by a baroreceptor reflex-mediated increase in locus coeruleus neuronal activity (Singewald *et al*., 1993).

Noradrenaline release in the prefrontal cortex was probably additionally decreased by presynaptic inhibition of noradrenaline release at the level of the axon terminals in the cortex. Clonidine and moxonidine, administered *via* microdialysis directly to the terminals of noradrenergic axons, inhibit the release of noradrenaline *in vivo* (e.g., Dalley & Stanford, 1995; van Veldhuizen *et al*., 1993; 1994). Clonidine and moxonidine also inhibit the release of noradrenaline from axon terminals in brain slices by a presynaptic mechanism (e.g., Limberger *et al*., 1989; Feuerstein & Limberger, 1999). The presynaptic inhibition of noradrenaline release in the cortex was probably weaker in our study than under truly physiological conditions, because the microdialysis fluid contained the noradrenaline uptake inhibitor desipramine and the calcium ion concentration was increased to 2.4 mmol l^{-1}. These modifications were necessary to facilitate the measurement of noradrenaline, but they are known to counteract the effects of presynaptic inhibitors (see Starke, 1987; Brock *et al*., 1990).

As mentioned above, it has been repeatedly shown that systemically administered clonidine inhibits the firing of locus coeruleus neurons (e.g., Svensson *et al*., 1975; Engberg & Eriksson, 1991; Berridge & Abercrombie, 1999). Systemically administered clonidine also decreased noradrenaline release, measured by microdialysis, in several brain regions (e.g., Abercrombie *et al*., 1988; Cuadra & Giacobini, 1995; Meana *et al*., 1997; Sacchetti *et al*., 1999). Simultaneous effects of clonidine on cerebral noradrenaline release and sympathetic tone have been determined in the present study for the first time.

Systemically administered rilmenidine lowers the firing rate of locus coeruleus neurons (Dresse & Scuvée-Moreau, 1986) but does not decrease the electrical stimulation-evoked release of noradrenaline in the hypothalamus (measured by voltammetry; Suaud-Chagny *et al*., 1992). The present study is the first in which the effect of rilmenidine on the activity of central noradrenergic neurons was evaluated with microdialysis and in which cardiovascular changes were recorded simultaneously. To our knowledge, the effects of systemically administered moxonidine and guanabenz on the activity of locus coeruleus neurons or on cerebral noradrenaline release have not been studied so far *in vivo*.

The main aim of the study was to compare the effects of the drugs on sympathetic tone with the effects on cerebrocortical noradrenaline release. Cortical noradrenaline release was not changed after the small doses of the drugs which produced only small and transient hypotension and bradycardia. Significant inhibition of cortical noradrenaline release was observed, however, after the higher doses which simultaneously decreased the plasma noradrenaline concen-

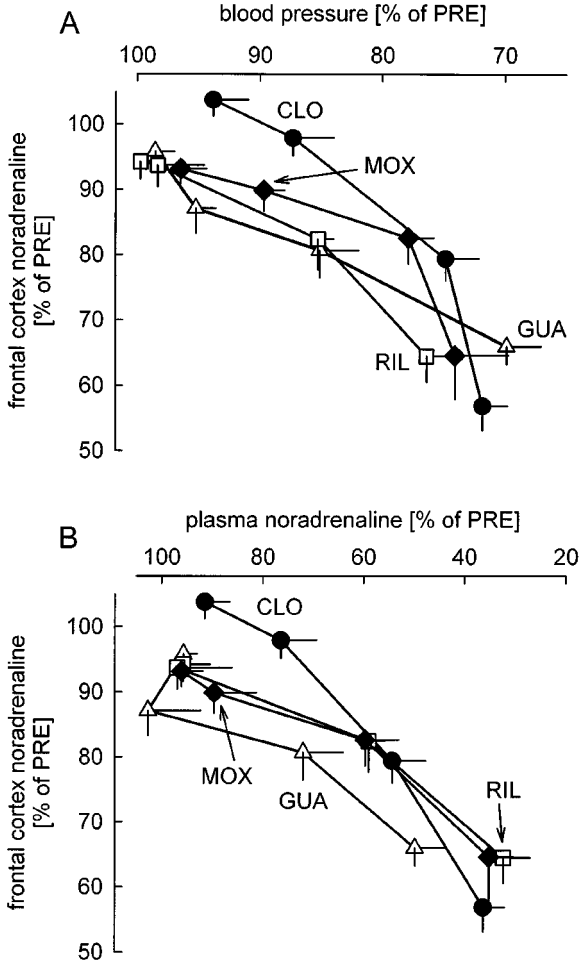

Figure 6 Correlation between effects of rilmenidine (RIL), moxonidine (MOX), clonidine (CLO) and guanabenz (GUA) on mean arterial pressure, the plasma noradrenaline concentration and the extracellular concentration of noradrenaline in the medial prefrontal cortex. The curves were constructed using the data shown in Figures 2–5. In the case of blood pressure, averages of the five values measured after administration of a drug dose were used.

tration, i.e., doses which caused sympatho-inhibition. Importantly, the two drugs with selectivity for imidazoline I_1 binding sites, rilmenidine and moxonidine (Ernsberger *et al*., 1993), also depressed cortical noradrenaline release. Rilmenidine and moxonidine were compared with clonidine and guanabenz, drugs which are not selective for I_1 binding sites (Ernsberger *et al*., 1993; Piletz *et al*., 1996). At doses eliciting equal hypotension and sympatho-inhibition, rilmenidine and moxonidine depressed cortical noradrenaline release at least as much as clonidine and guanabenz.

Our finding – sympatho-inhibitory doses of imidazolines inhibit the activity of locus coeruleus neurons – are in accordance with several previous studies. Thus, clonidine and rilmenidine lowered the firing rate of locus coeruleus neurons with ED_{50} values of 5–8 μg kg^{-1} (i.v.) and 350 μg kg^{-1} (i.v.), respectively (Dresse & Scuvée-Moreau, 1986; Engberg & Eriksson, 1991). Somewhat higher doses were necessary to produce significant inhibition of cortical noradrenaline release in the present study, probably because microdialysis is not sensitive enough to detect small decreases in noradrenaline release lasting shorter than the 25-min sampling period. Li & Dampney (1995) determined the expression of Fos protein, a marker of neuronal activation, immunohistochemically. Infusion of sodium nitroprusside led to Fos expression in the RVLM and the locus coeruleus. Clonidine (7–30 μg kg^{-1}, i.v.) and rilmenidine (150–300 μg kg^{-1}, i.v.) suppressed the appearance of Fos protein in the RVLM as well as in the locus coeruleus; importantly, no preferential effect of the drugs in the RVLM was seen.

Our finding stands in contrast to previous results obtained with voltammetry. Rilmenidine (7.5 mg kg^{-1}; i.v.) had no effect on locus coeruleus neuronal activity as estimated by voltammetric measurement of the concentration of dihydroxyphenylacetic acid in the locus coeruleus (Tibiriça *et al*., 1991). In another study, rilmenidine (3 and 10 mg kg^{-1}; i.v.) even potentiated the increase in the voltammetric signal (attributed to noradrenaline) in the hypothalamus evoked by electrical stimulation of the afferent noradrenergic pathway (Suaud-Chagny *et al*., 1992). In this latter publication, it was concluded that 'rilmenidine, at clinically relevant doses, is not active on central α_2-adrenoceptors'. These findings are clearly in contrast to the present findings in which rilmenidine, at doses of 0.3–1 mg kg^{-1} (i.v.), lowered the activity of neurons projecting from the locus coeruleus to the medial prefrontal cortex. We can only speculate on the reason for the discrepancy. DOPAC may be a suboptimal indicator of the activity of noradrenergic neurons. The release of noradrenaline in the hypothalamus was evoked by stimulation at high frequency (10 Hz; Suaud-Chagny *et al*., 1992); presynaptic inhibition is weak under this condition (see Starke, 1987).

It was not the aim of our study to characterize the receptors which mediate the depression of prefrontal cortex noradrenaline release. However, several arguments support the view that they were α_2-adrenoceptors of the $\alpha_{2A/D}$ subtype. Imidazoline receptors responsive to clonidine, rilmenidine and moxonidine were not found in the locus coeruleus (Szabo *et al*., 1996). Clonidine and rilmenidine excite locus coeruleus neurons *in vivo* through an imidazoline receptor-mediated action in the paragigantocellular nucleus; but this effect occurs only at high doses of the drugs and only in the presence of α_2-adrenoceptor blockade (Pineda *et al*., 1993; Ruiz-Ortega & Ugedo, 1997; see also Meana *et al*., 1997). Messenger RNA and receptor protein for $\alpha_{2A/D}$-adrenoceptors (but also for α_{2C}-adrenoceptors) were demonstrated in the locus coeruleus (Nicholas *et al*., 1996; Rosin *et al*., 1996; Talley *et al*., 1996). The α_2-adrenoceptor responsible for the inhibition of locus coeruleus neurons and sedation was identified as the $\alpha_{2A/D}$ receptor (Chiu *et al*., 1995; Mizobe *et al*., 1996; Nörenberg *et al*., 1997; Mateo & Meana, 1999). The common property of the four drugs used by us is their agonist activity at α_2-adrenoceptors. Three of the drugs, clonidine, rilmenidine and moxonidine in fact were shown to inhibit the firing of locus coeruleus neurons in brain slices by activating α_2-adrenoceptors (Williams *et al*., 1985; Szabo *et al*., 1996; see above). The presynaptic receptors at the terminals of noradrenergic axons in the prefrontal cortex were probably also $\alpha_{2A/D}$-adrenoceptors, since these are the predominant α_2-adrenoceptors mediating presynaptic inhibition of noradrenaline release in cortical brain slices (Trendelenburg *et al*., 1999). Moreover, no presynaptic imidazoline receptors were found on noradrenergic axons in the cortex (Gaiser *et al*., 1999).

At doses lowering blood pressure of hypertensive patients equally, rilmenidine and moxonidine cause less sedation than clonidine; the difference is prominent at the beginning of the treatment but disappears during several weeks of therapy (Plänitz, 1984; 1987; Fillastre *et al*., 1988). The imidazoline hypothesis offered an explanation for the low incidence of sedation: due to their high affinity for I_1 imidazoline receptors and lower affinity for α_2-adrenoceptors rilmenidine and moxonidine lower sympathetic tone without activating 'sedative' α_2-adrenoceptors in the locus coeruleus. The present results contradict this explanation. At sympatho-inhibitory doses, rilmenidine and moxonidine depressed the activity of noradrenergic locus coeruleus neurons, and they depressed it at least as much as clonidine. Therefore, it remains open why rilmenidine and moxonidine, at least at the beginning of therapy, cause less sedation than clonidine.

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