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Pre- and postsynaptic inhibitory potencies of the angiotensin AT_1 receptor antagonists eprosartan and candesartan

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Abstract

The aim of the present study was to determine the inhibitory potency of two selective angiotensin AT_1 receptor antagonists, eprosartan and candesartan, at the level of the sympathetic nerve terminal and the vascular smooth muscle. Male New Zealand White rabbits, weighing 2100–2550 g, were used. To study eprosartan and candesartan at the neuronal angiotensin AT_1 receptor, we investigated their influence on the angiotensin II-enhanced, electrical field stimulation-evoked sympathetic transmission in the rabbit isolated thoracic aorta in a noradrenaline spillover model. To study both antagonists at the vascular angiotensin AT_1 receptor, concentration–response curves for angiotensin II were constructed in the presence or absence of the two angiotensin AT_1 receptor antagonists. Angiotensin II (10 nM) caused a significant increase by $107 \pm 11.1\%$ of the stimulation-evoked sympathetic outflow, which was concentration-dependently inhibited by both eprosartan (pIC_{50} 7.91 ± 0.12) and candesartan (pIC_{50} 10.76 ± 0.13). Angiotensin II (1 nM–0.3 μM) caused a concentration-dependent increase in contractile force (E_{max} 20.62 ± 2.24 mN, pD_2 8.16 ± 0.04). Both eprosartan (pA_2 8.90 ± 0.11, pIC_{50} 8.87 ± 0.12 (10 nM angiotensin II)) and candesartan (pD'_2 10.80 ± 0.13) counteracted the contractions evoked by cumulative concentrations of angiotensin II. Candesartan proved a more potent antagonist than eprosartan at both the pre- and postjunctional angiotensin AT_1 receptor. For eprosartan, vascular inhibitory concentrations were 10-fold lower than sympatho-inhibitory concentrations, whereas for candesartan, inhibitory concentrations at both sites were similar. The results may be explained by differences between the pre- and postjunctional angiotensin AT_1 receptor subtype.

Keywords: Angiotensin II; Renin–angiotensin system; Angiotensin AT_1 receptor antagonist; (Rabbit)

1. Introduction

Both the sympathetic nervous system and the renin–angiotensin system offer major targets for pharmacological interventions in cardiovascular diseases. Several studies have revealed that angiotensin II interacts with the sympathetic nervous system at several sites, including the central nervous system, adrenal medulla, sympathetic ganglia and post-ganglionic nerve terminals (Reid, 1992). At the sympathetic nerve terminals, angiotensin II influences sympathetic transmission by blocking noradrenaline uptake, enhancing noradrenaline synthesis and facilitating noradrenaline release.

Angiotensin II can stimulate at least two pharmacologically distinct receptors. Receptor binding studies differentiate between the angiotensin AT_1 receptor (antagonized by losartan) and the angiotensin AT_2 receptor (antagonized by PD123319; (*S*)-1-([4-(dimethylamino)-3-methylphenyl]-methyl)-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo(4,5-*c*]pyridine-carboxylic acid, ditrifluoroacetate, dihydrate) (Chiu et al., 1989; Dudley et al., 1990;Whitebread et al., 1989). Effects mediated by the angiotensin AT_1 receptor include a direct vasoconstriction, vascular hypertrophy (Chiu et al., 1991) and secretion of aldosterone by the adrenal cortex (Wong et al., 1990), actions that are known to be detrimental in a setting of hypertension or heart failure. By contrast, the angiotensin AT_2 receptor may oppose these angiotensin AT_1 receptor-mediated actions (Burnier, 2001). Additionally, the angiotensin AT_1 receptor subtype is assumed to mediate the enhancement of sympathetic neurotransmission (Brasch et al., 1993; Hegde and Clarke, 1993; Tofovic et al., 1991).

Recently, several authors have compared the sympatho-inhibitory potency of various angiotensin AT_1 receptor antagonists (Balt et al., 2001a; Dendorfer et al., 2002; Nap et al., 2002). Significant differences were repeatedly des-

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cribed, indicating affinity differences for the prejunctional angiotensin receptor between various blockers. Sympatho-inhibition, however, appears to be a class effect since all angiotensin AT_1 receptor antagonists investigated attenuated the angiotensin II-enhanced sympathetic neurotransmission to the same maximal degree. These findings provide strong evidence supporting the concept that enhancement of sympathetic transmission at the prejunctional level is mediated by the angiotensin AT_1 receptor subtype.

Discrepancies regarding the potency in inhibiting neuronal and vascular effects of angiotensin II have been described for several angiotensin AT_1 receptor antagonists. It appears that these potency differences might be explained by differences in the characteristics of the pre- and postsynaptic angiotensin AT_1 receptor, respectively (Balt et al., 2001b; Guimaraes et al., 2001).

Several studies that assessed the prejunctional effects of angiotensin II were based on the quantification of postsynaptic effects of the released transmitter, whereas quantification of sympathetic transmitter release (noradrenaline spillover) offers a more straightforward approach. In addition, prejunctional inhibitory potencies were obtained mostly using different concentrations angiotensin II compared to postjunctional inhibitory potencies. Accordingly, definite data concerning a possible neuronal or vascular selectivity of the clinically used angiotensin AT_1 receptor antagonists, which in addition may be explained by angiotensin AT_1 receptor subtype differences, is limited.

For these reasons, we studied the neuronal and vascular inhibitory potencies of two angiotensin AT_1 receptor antagonists. We deliberately compared eprosartan and candesartan, since candesartan displayed but weak sympatho-inhibitory activity compared to its potent action at the postsynaptic angiotensin AT_1 receptor, whereas for eprosartan, sympatho-inhibitory dosages were similar to those required for antagonism of angiotensin II-induced vasoconstriction (Balt et al., 2001b). To overcome the limitations of previous studies, we investigated the sympatho-inhibitory potencies of eprosartan and candesartan on angiotensin II-enhanced, electrical field stimulation-evoked sympathetic transmission in the rabbit thoracic aorta in a noradrenaline spillover model. To compare the inhibitory potencies on the vasculature, concentration–response curves for angiotensin II were constructed in the presence or absence of the angiotensin AT_1 receptor antagonists. Additionally, we used equivalent concentrations angiotensin II to compare the pre- and postjunctional inhibitory concentrations (pIC_{50}) of the selective angiotensin AT_1 receptor antagonists.

2. Material and methods

Experiments were performed with thoracic aortic rings from male New Zealand White rabbits, weighing 2100–2550 g, and obtained from the Common Animal Institute Amsterdam. The committee for Animal Experiments of the Academic Medical Center Amsterdam has approved the experimental protocol. The investigation conforms with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH 85-23, revised 1996).

The rabbits were anaesthetized with Hypnorm (fentanyl/fluanison 2.5 mg/kg, i.m.), injected with heparin 875 IE/kg i.v. and subsequently sacrificed with Nembutal (pentobarbital 30 mg/kg, i.v.). The thoracic cavity was opened and, after removal of the heart and lungs, the thoracic aorta was dissected free from its connective tissue and transferred to a physiological salt solution, oxygenated by carbogen (95% O_2 + 5% CO_2) and kept at room temperature. Rings of thoracic aorta were prepared and placed in an organ bath setup. The medium was composed of (mmol/l): NaCl 118, Na_2HPO_4 1.2, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 1.6, $MgSO_4$ 1.2, and glucose 11.0. Ascorbic acid (0.3) and Na_2 EDTA (0.03) were added to prevent oxidation of noradrenaline.

2.1. Experiments concerning sympathetic outflow

These experiments were performed using a modified spillover model, allowing the selective measurement of sympathetic outflow using tritium-labeled noradrenaline (Nap et al., 2001).

2.1.1. Radiolabelling of noradrenergic transmitter stores

In order to label their noradrenergic transmitter stores, the aortic rings were incubated for 45 min in 2.0 ml of physiological salt solution containing 0.1 μmol/l L-[7,8-^{3}H]noradrenaline (specific activity 28.8–52.0 Ci/mmol) in a 5-ml glass-jacketed organ bath. The medium was continuously bubbled with carbogen and maintained at a temperature of 37 °C.

After the incubation period, the isolated aortic rings were washed with [^{3}H]noradrenaline-free physiological salt solution (10 × 2 ml and 4 × 5 ml) to remove superficially bound, non-neuronal radioactivity before the experimental procedures were started, and mounted vertically between platinum wire electrodes (2 cm) placed along either side of the preparations in a 25-ml organ bath and subjected to a tension of 0.5 g. The organ bath contained 20.0 ml physiological salt solution. Desipramine (0.6 μmol/l) and corticosterone (40 μmol/l) were added to rule out uptake-1 and uptake-2 of [^{3}H]noradrenaline, respectively. Yohimbine (1 μmol/l) was added to rule out any α_2-adrenergic auto-inhibitory effects on [^{3}H]noradrenaline release. The aortic rings were equilibrated for a total of 48 min. After an initial period of 18 min, the preparations were subjected to a 2-min period of EFS with a train of 3 ms rectangular bipolar wave pulses of 150 mA, at a frequency of 2 Hz (S_0) (Danish Myo Technology Current Stimulator, model CS 200). This 'priming' stimulation has proven to increase the reliability and stability of the subsequent basal and electrical field stimulation-induced [^{3}H]noradrenaline spillover.

2.1.2. Stimulation of intrinsic sympathetic nerves

After the equilibration period, the aortic preparations were subjected to two periods of electrical field stimulation (see above). The first period of stimulation (S_1) was applied directly after the equilibration period of 48 min and the radioactivity thus evoked was taken as control value. Subsequently, a second period (S_2) was applied 24 min after S_1.

2.1.3. Measurement of tritium-label

Samples of 0.5 ml were repeatedly taken from the organ bath starting 36 min after washout. Since the organ bath medium was not changed and its total volume decreased stepwise by repeated drawing of samples, the actual outflow of radioactivity could be obtained by calculating the incremental accumulation in each sample corrected for the reduced volume.

The mean basal efflux of radioactivity/min preceding S_1 and S_2 was determined as the mean outflow/min of radioactivity in two 6-min samples prior to each period of stimulation. The release/min evoked by electrical field stimulation (S_1 and S_2, 2-min samples) was calculated by subtracting the corresponding mean basal efflux/min from the apparent evoked efflux/min. At the end of the experiment, the residual radioactivity of the tissue was measured. By adding the total released radioactivity, the initial content of tritium-label was calculated. The effect of electrical field stimulation on the release could then be expressed as a fraction of the total tissue content present at the time at which the stimulation period was applied or 'fractional release' of radioactivity (FR_1 and FR_2). Accordingly, the effects of pharmacological interventions are expressed as the ratio FR_2/FR_1.

2.1.4. Detection of tritium-label in the samples and tissue

After the experiment, the tissues were kept overnight in 2 ml Soluene. Radioactivity was measured by liquid scintillation counting (Tri Carb 2900TR, Packard) in 20-ml aliquots (with either samples or tissue) after addition of 5 ml of scintillation mixture (Ultima Gold). Corrections for counting efficiency were made by external automatic standardization.

2.1.5. Influence of selective angiotensin AT_1 receptor antagonists on angiotensin II-enhanced, stimulation-evoked sympathetic outflow

To investigate the influence of angiotensin II (10 nM) on electrical field stimulation-evoked noradrenaline release, it was added to the medium 150 s prior to S_2. In another group of preparations, the influence of multiple concentrations of eprosartan (0.1 nM–0.1 μM) and candesartan (1 pM–0.1 nM) on angiotensin II-enhanced sympathetic outflow was investigated. Eprosartan and candesartan were added to the medium in one particular concentration 20 min before S_2. To indicate the effects of the applied compounds, the ratio FR_2/FR_1 was used.

2.2. Experiments concerning postjunctional actions of angiotensin II

The aortic rings were mounted between two triangular stainless steel hooks and placed into an organ bath setup with isometric tension recording. The medium, consisting of 5 ml physiological salt solution, was continuously bubbled with carbogen and maintained at a temperature of 37 °C. N^{ω}-nitro-L-arginine (L-NNA, 0.1 mM) was added to exclude the influence of endothelium-derived nitric oxide.

Isometric tension was measured by means of isometric force transducers (A.D. Instruments, Castle Hill, Australia), connected to a MacLab/8 computer system. The aortic rings were equilibrated for 30 min at a resting tension of 20 mN, which was maintained throughout the experiment. The equilibration period was followed by a priming procedure that consisted of a single application of 60 mM K^+ (physiological salt solution containing 60 mM KCl; equimolar substitution for NaCl) of 3 min, a single concentration of angiotensin II (0.1 μM), followed by two subsequent 60 mM K^+-induced depolarizations. Each stimulus was applied 15 min after the preparations had been washed out repeatedly and had returned to a resting tension of 20 mN.

2.2.1. Influence of selective angiotensin AT_1 receptor antagonists on the contraction elicited by angiotensin II

Concentration–response curves for angiotensin II (1 nM–0.3 μM) were obtained by single additions with half-log increments 35 min subsequent to the priming procedure. To investigate the influence of eprosartan and candesartan on angiotensin II-elicited contractions, a concentration–response curve for angiotensin II (1 nM–0.03 mM) was constructed in the presence of one particular concentration of the angiotensin AT_1 receptor antagonist to be tested, or the vehicle. The antagonists were added to the medium 20 min before the concentration–response curve was started. Using a computer program (GraphPad Prism, GraphPad, San Diego, CA, USA), all curves were fitted to log concentration–effect data. The underlying equation is $E = E_{max}A^p * (A^p + IC_{50}^p)^{-1}$. In this equation, E is the response obtained at a given concentration A, E_{max} is the maximally attainable response, IC_{50} the concentration antagonist for the half-maximal effect, and the exponent p describes the slope of the relationship (Hill coefficient). EC_{50} represents the concentration of angiotensin II that caused half-maximal effects in the absence of blocker. Curves were fitted to averaged concentration–effect data. The effects of the antagonists were expressed as percentage of the maximum contractile force elicited by angiotensin II alone (%E_{max}).

2.2.2. Influence of angiotensin II on the contraction elicited by noradrenaline

Concentration–response curves for noradrenaline (1 nM–0.1 mM), in the presence of angiotensin II (1 nM, sub-pressor concentration, added 2 min before the concentration–response curve was started) or the vehicle, were

obtained by single additions with half-log increments 35 min subsequent to the priming procedure. The results were calculated and expressed as pointed out for experiment 2.

2.3. Drugs and chemicals

Desipramine HCl, yohimbine HCl and N^{ω}-nitro-L-arginine (Sigma, USA) were dissolved in distilled water. Corticosterone (Bufa, The Netherlands) was dissolved in dimethylsulfoxide (DMSO). Stock solutions of desipramine (0.6 mM), yohimbine (1 mM) and corticosterone (40 mM) were further diluted with physiological salt solution.

Angiotensin II (Bachem, Bubendorf, Switzerland, synthetic human sequence) was dissolved in distilled water. Stock solutions of angiotensin II (0.1 mM) were stored in 50-μl aliquots at −20 °C. Tritiated L-[7,8-^{3}H]noradrenaline (Amersham Pharmacia Biotech, Little Chalfont, England) had a specific radioactivity of 28.8–52.0 Ci/mmol and a radioactive concentration of 1.0 mCi/ml. Soluene and Ultima Gold solutions were obtained from Packard (Groningen, The Netherlands). Eprosartan (Solvay, Hannover, Germany) and candesartan (AstraZeneca, Södertälje, Sweden) were dissolved in 1 M NaOH. Using 1 M HCl, the pH of the solutions was adjusted to 7.5. (−)-Noradrenaline bitartrate (Sigma) was dissolved in distilled water containing 1 mg/ml L-(+)-ascorbic acid.

2.4. Statistical analysis

All data are expressed as means ± S.E.M. Student's *t*-test (two-tailed, unpaired) was used to evaluate statistical significance of differences between means of control and treatment groups. An analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test was used for multiple comparisons with a control group. Differences at $P<0.05$ were considered to indicate statistical significance.

The degree of inhibition by the different concentrations of angiotensin AT_1 receptor antagonists was determined by subtracting the individual response from the average response seen in the presence of angiotensin II alone and by dividing that value by the average net increase effected by angiotensin II relative to the average control response:

$$\text{Fraction inhibition} = \frac{[\overline{X}_{(A)} - X_{i(AB)}]}{[\overline{X}_{(A)} - \overline{X}_{(C)}]} \quad (1)$$

where A = angiotensin II (10 nM), B = angiotensin AT_1 receptor antagonist and C = control.

In order to compare the sympatho-inhibitory potency of both eprosartan and candesartan, we calculated the pIC_{50} (concentration antagonist that caused 50% reduction in the response elicited by angiotensin II (10 nM).

In order to compare the potency of the angiotensin AT_1 receptor antagonists concerning inhibition of vasoconstrictor responses elicited by angiotensin II, pA_2 values (concentration antagonist that causes a right-shift of a factor 2 of the concentration–response curve for angiotensin II) and/or pD_2' values (concentration antagonist that causes 50% reduction of E_{max}) were calculated. Additionally, the IC_{50} values (the concentration angiotensin AT_1 receptor antagonist that causes 50% reduction in angiotensin II-mediated responses) were determined. Differences with $P<0.05$ were considered significant. Linear regression was performed and analysis of covariance was used to evaluate differences between regression lines. Differences at $P<0.05$ were considered to indicate statistical significance.

3. Results

3.1. Influence of selective angiotensin AT_1 receptor antagonists on angiotensin II-enhanced sympathetic outflow, evoked by electrical field stimulation

In control experiments, we observed no change of the basal efflux of radioactivity between the different periods of

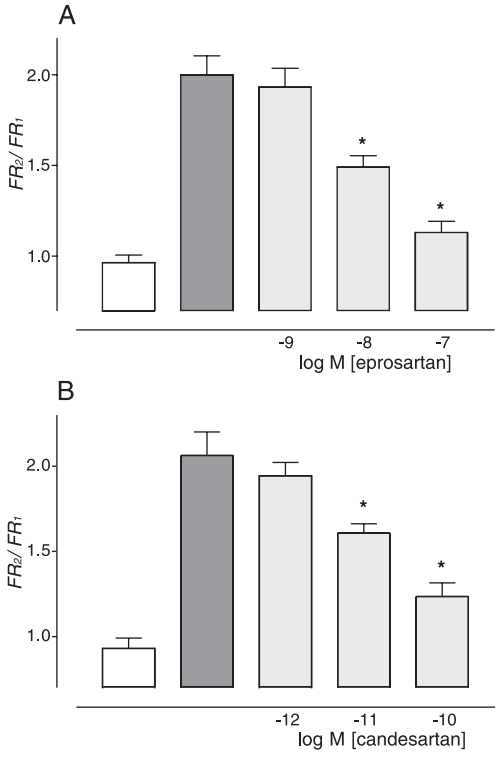

Fig. 1. Inhibitory effects of eprosartan (1 nM–0.1 μM) (A) and candesartan (1 pM–0.1 nM) (B) on the facilitation by angiotensin II of electrical field stimulation-evoked [^{3}H]noradrenaline outflow from isolated rabbit thoracic aortic rings. The ring preparations were stimulated at 24-min intervals. Angiotensin II (10 nM) in the presence or absence of the two selective angiotensin AT_1 receptor antagonists was added to the organ bath 150 s before S_2. The antagonists were added 20 min prior to S_2. The ratio between fractional releases evoked by S_2 (FR_2) and S_1 (FR_1) is shown on the vertical axis, the concentrations of the angiotensin AT_1 receptor antagonists (expressed as log M) on the horizontal axis. □, control; ■, angiotensin II; ■, angiotensin II + antagonist. Columns represent means ± S.E.M. Asterisk indicates $P<0.05$ compared with angiotensin II group (ANOVA followed by Bonferroni's test, n = 6–7 per group).

stimulation. Stimulation of the aortic rings resulted in a marked increase in sympathetic outflow by approximately a factor 6 ($P<0.05$, data not shown). Furthermore, the fractional release of stimulation-evoked radioactivity remained constant throughout the experiment (FR_2/FR_1: 0.96 ± 0.04, $n=8$).

To validate that the evoked tritium-label spillover is of neuronal origin, we tested the influence of tetrodotoxin (1 μM) on stimulation-evoked sympathetic outflow. Tetrodotoxin nearly abolished the evoked release of radioactivity (data not shown).

Angiotensin II (10 nM) added to the organ bath 150 s before S_2 did not alter the efflux at rest. However, it caused a significant increase by approximately 107% of the stimulation-evoked sympathetic outflow (FR_2/FR_1: 2.00 ± 0.11, $n=10$, $P<0.05$).

Addition of eprosartan and candesartan to the medium 20 min before S_2 neither influenced the resting efflux nor the tritium-label spillover evoked by electrical field stimulation (data not shown). However, both eprosartan (1 nM–0.1 μM) and candesartan (1 pM–0.1 nM) concentration-dependently attenuated the subsequent angiotensin II-enhanced (10 nM) sympathetic outflow (Fig. 1A and B). The lowest concentrations of eprosartan (1 nM) and candesartan (1 pM) proved not to be inhibitory; conversely, the higher concentrations of the angiotensin AT_1 receptor antagonists significantly attenuated the angiotensin II-enhanced (10 nM) sympathetic outflow as compared to the responses in the presence of angiotensin II alone ($P<0.05$).

The fraction of inhibition (see Eq. (1)) displayed by both blockers is shown in Fig. 2. A linear correlation was observed between the fraction of inhibition and the different concentrations of the angiotensin AT_1 receptor antagonists applied. The IC_{50} value of eprosartan (pIC_{50} 7.91 ± 0.12) differed significantly ($P<0.05$) from that of candesartan (pIC_{50} 10.76 ± 0.13). In addition, the regression lines of eprosartan and candesartan differed significantly ($P<0.05$). For eprosartan: slope = 0.3861 ± 0.05242; *Y*-intercept = 3.552 ± 0.4215; *X*-intercept = -9.201. For candesartan: slope = 0.3219 ± 0.04449; *Y*-intercept = 3.965 ± 0.4907; *X*-intercept = -12.32.

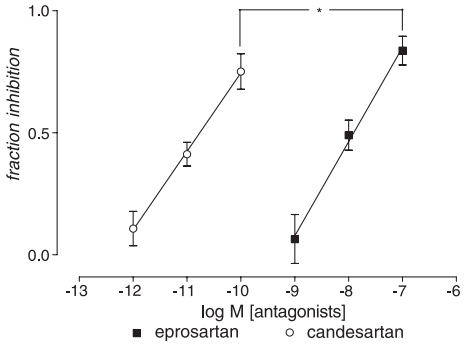

Fig. 2. Fraction of inhibition caused by eprosartan and candesartan on the enhancement by angiotensin II (10 nM) of electrical field stimulation-evoked [^{3}H]noradrenaline outflow from isolated rabbit thoracic aortic rings. Linear regression analysis adequately described the relation between fraction of inhibition and concentrations of the angiotensin AT_1 receptor antagonists applied. Angiotensin II (10 nM) was added to the organ bath 150 s before S_2. The antagonists were added 20 min prior to S_2. The fraction inhibition is shown on the vertical axis. The concentrations of the angiotensin AT_1 receptor antagonists (expressed as log M) are expressed on the horizontal axis. Values are expressed as means ± S.E.M. Asterisk indicates $P<0.05$ (ANOVA followed by Bonferroni's test, $n=6-7$ per group).

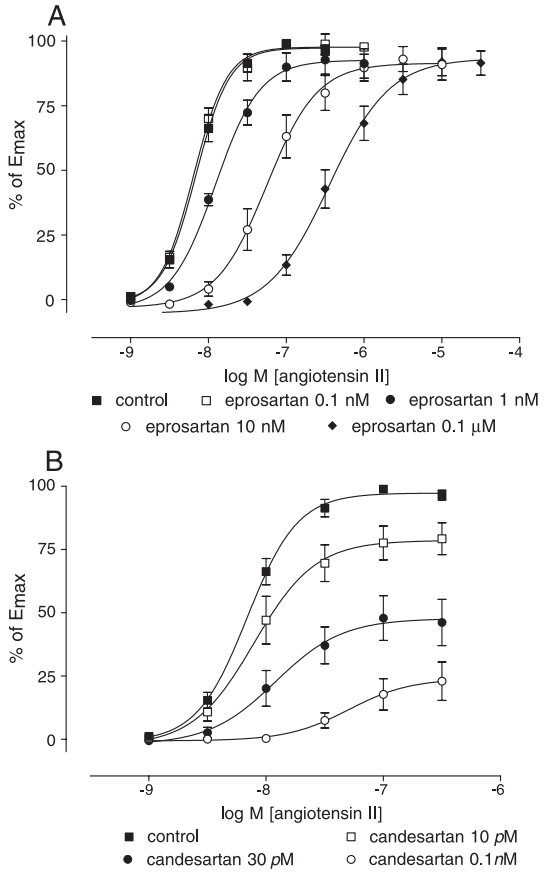

Fig. 3. Effects of the selective angiotensin AT_1 receptor antagonists eprosartan (0.1 nM–0.1 μM) (A) and candesartan (0.01 nM–0.1 nM) (B) on the constrictor response induced by angiotensin II in isolated rabbit thoracic aortic rings. The antagonists were added 20 min before the concentration–response curve was started. The developed force, expressed as percentage of the maximum contractile force elicited by angiotensin II ($\%E_{max}$) is shown on the vertical axis. The concentrations angiotensin II (expressed as log M) are expressed on the horizontal axis. Values are expressed as means ± S.E.M., $n=6-7$ per group.

3.2. Influence of selective angiotensin AT_1 receptor antagonists on the contraction elicited by angiotensin II

Angiotensin II (1 nM–0.3 μM) caused a concentration-dependent increase in contractile force of rabbit thoracic aortic rings (E_{max} 20.62 ± 2.24 mN, pD_2 8.16 ± 0.04, $n=10$). Both eprosartan (0.1 nM–0.1 μM) and candesartan (0.01–0.1 nM) influenced the contractions to cumulative concentrations of angiotensin II (Fig. 3A and B). Eprosartan inhibited angiotensin II-induced contractions in a manner that is consistent with competitive antagonism (pA_2

8.90 ± 0.11, $n = 24$). Additionally, we calculated a pIC_{50} of 8.87 ± 0.12 in the presence of 10 nM angiotensin II. Candesartan inhibited angiotensin II-induced contractions in a manner that is consistent with noncompetitive antagonism (pD_2' 10.80 ± 0.13, $n = 21$).

3.3. Influence of angiotensin II on the contraction elicited by noradrenaline

Noradrenaline caused a concentration-dependent increase in contractile force (EC_{50} -6.95 ± 0.05 log M, E_{max} $129.8 \pm 2.0\%$ of contractions by 60 mM K^+ (E_{max} 36.85 ± 1.07 mN), $n = 4$). Vasoconstrictor responses to noradrenaline remained unchanged by the presence of 1 nM angiotensin II (EC_{50} -6.93 ± 0.05 log M, E_{max} $129.7 \pm 2.2\%$ of contractions by 60 mM K^+ (E_{max} 35.10 ± 1.27 mN)). $P > 0.05$ compared to control for both E_{max} and EC_{50} values.

4. Discussion

The sympatho-inhibitory properties of the selective angiotensin AT_1 receptor antagonists eprosartan and candesartan were determined and compared with their potency in inhibiting the constrictor effects of angiotensin II on the vasculature. Candesartan proved more potent at either site. Differences between pre- and postjunctional inhibition were observed for eprosartan, which may be explained by differences between the pre- and postjunctional angiotensin AT_1 receptor subtype.

4.1. Experiments concerning sympathetic outflow

Eprosartan and candesartan concentration-dependently inhibited angiotensin II-enhanced sympathetic transmission in the rabbit thoracic aorta (Fig. 1A and B), thus confirming that the presynaptic angiotensin II receptor belongs to the angiotensin AT_1 receptor subtype. Sympatho-inhibitory concentrations differed significantly between eprosartan (pIC_{50} 7.91 ± 0.12) and candesartan (pIC_{50} 10.76 ± 0.13), whereas the degree of inhibition provoked by each angiotensin AT_1 receptor antagonists was similar (Fig. 2). Accordingly, on a molar basis, candesartan is more potent than eprosartan. Differences in sympatho-inhibitory potency suggest that the antagonists display different affinities for the prejunctional angiotensin AT_1 receptor. Furthermore, as indicated earlier (Balt et al., 2001a; Nap et al., 2002), sympatho-inhibition appears to be a class effect of the selective angiotensin AT_1 receptor antagonists.

The sympatho-inhibitory concentration range of eprosartan was similar to that applied in other experiments (Guimaraes et al., 2001). The observed potent sympatho-inhibitory properties of candesartan, however, are unexpected, since in the pithed rat model candesartan proved significantly less potent than eprosartan (Balt et al., 2001b). These differences may be explained by pharmacokinetic characteristics associated with the in vivo model. However, the elimination half-life of candesartan exceeds that of eprosartan and therefore cannot account for the discrepancies (Burnier, 2001; Hubner et al., 1997; Bottorff and Tenero, 1999). Plasma protein binding of candesartan amounts to 99.4–99.8% (van Lier et al., 1997), whereas eprosartan binds for 98% (Burnier, 2001; Martin et al., 1998). This apparent small difference results in a 3- to 10-fold higher free fraction of eprosartan, which may account for the observed order in the pithed rat model. Additionally, a high affinity and/or slow dissociation from the receptor-binding site (Ojima et al., 1997) may explain the potent sympatho-inhibitory properties of candesartan displayed in the current investigation in vitro.

4.2. Experiments on postjunctional actions of angiotensin II

Eprosartan and candesartan both concentration-dependently inhibited the constrictor response to angiotensin II (Fig. 3A and B). Eprosartan did so in a manner that is consistent with competitive antagonism (pA_2 8.90 ± 0.11). Potent competitive antagonism (nM range) of eprosartan has been observed in the rabbit thoracic aorta (Edwards et al., 1992) and in renal arterioles (Edwards and Aiyar, 1993). In contrast, candesartan inhibited angiotensin II-induced contractions in a manner that is consistent with noncompetitive antagonism (pD_2' 10.80 ± 0.13). Highly potent, noncompetitive antagonism (IC_{50} 10^{-10} M) has been observed in the rabbit thoracic aorta (Nishikawa et al., 1994; Noda et al., 1993; Shibouta et al., 1993). Accordingly, in conformity with previous in vitro studies, candesartan appears to be a more potent selective angiotensin AT_1 receptor antagonist compared to eprosartan regarding the vascular angiotensin AT_1 receptor. In addition, these results once more confirm that the postjunctional angiotensin II receptor belongs to the angiotensin AT_1 receptor subtype.

No differences were observed between the vasoconstrictor responses to exogenous noradrenaline in the presence or absence of 1 nM angiotensin II, which proved to significantly enhance EFS-evoked noradrenaline outflow (Nap et al., 2002). Therefore we could not confirm a facilitatory role of angiotensin II on postsynaptic α-adrenoceptor-mediated responses, as reported by others (Purdy and Weber, 1988; Tofovic et al., 1991). In the rat mesenteric artery and the rabbit ear artery, as in the present study, no effect of angiotensin II on noradrenaline responses was observed (Balt et al., 2001a; Duckles, 1981).

4.3. Neuronal and vascular angiotensin AT_1 receptor diversity?

Recently, several studies have addressed the issue of vascular and neuronal angiotensin AT_1 receptor subtype differences (Dendorfer et al., 2002; Balt et al., 2001b; Guimaraes et al., 1998, 2001). The potencies of several

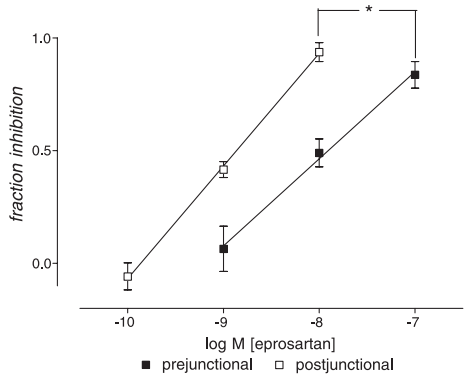

Fig. 4. Fraction of inhibition caused by eprosartan on the enhancement by angiotensin II (10 nM) of stimulation-evoked [^{3}H]noradrenaline outflow and on the angiotensin II-mediated (10 nM) constrictor response of isolated rabbit thoracic aortic rings. Linear regression analysis adequately described the relation between fraction of inhibition and concentrations of eprosartan applied. The fraction inhibition is shown on the vertical axis. The concentrations of eprosartan (expressed as log M) are expressed on the horizontal axis. Values are expressed as means ± S.E.M. Asterisk indicates $P < 0.05$.

selective angiotensin AT_1 receptor antagonists at the level of the varicosity and the vasculature differed significantly in a pithed rat study by Balt et al. (2001b). In the canine pulmonary artery and rat left ventricle, differences in the prejunctional and postjunctional inhibitory potency of losartan were described by Guimaraes et al. (2001). As designated by both studies, differences in receptor subtype between the pre- and postjunctional angiotensin AT_1 receptor may explain these observations.

More recently, however, this hypothesis was challenged by Dendorfer et al. (2002), who demonstrated similar sympatho-inhibitory and vascular inhibitory potencies for several selective angiotensin AT_1 receptor antagonists in the pithed rat. By infusing exogenous angiotensin II, an increase in plasma noradrenaline was observed, which was inhibited by selective angiotensin AT_1 receptor blockade. These authors, however, did not apply electrical field stimulation of the thoracolumbar spinal cord and therefore did not describe prejunctional facilitation of depolarization-induced catecholamine release. Their observations may be explained by direct angiotensin II-mediated ganglionic excitation, as was reported by Ma et al. (2001) in mice renal sympathetic nerves. As a further limitation, no bilateral adrenalectomy was performed and, hence, the increase in plasma noradrenaline levels after infusion of angiotensin II can, at least partially, be explained by catecholamine release from the adrenal medulla.

In the present study, we compared the pre- and postjunctional inhibitory potency of two angiotensin AT_1 receptor antagonists. Candesartan displayed similar inhibitory potencies at both levels (pIC_{50} 10.76 ± 0.13 neuronal, pD_2' 10.80 ± 0.13 vascular). For eprosartan, we assessed the pIC_{50} in the presence of the same concentration of angiotensin II (10 nM) at the sympathetic nerve terminal and the vasculature (Fig. 4). Accordingly, the vascular pIC_{50} amounted to 8.87 ± 0.12, which differed approximately by 10-fold from the neuronal pIC_{50} (7.91 ± 0.12) of eprosartan. These results may be explained by subtype differences between the prejunctional and postjunctional angiotensin AT_1 receptor.

The sympathetic nervous system and the renin–angiotensin system are involved in important cardiovascular diseases (Mark, 1996; Mancia, 1990), therefore both pre- and postjunctional inhibitory properties, as evaluated in the current study, may be clinically relevant. It seems likely that part of the pressor response to angiotensin II is evoked by angiotensin II-mediated enhancement of sympathetic transmission. Since the sympatho-inhibitory concentrations of eprosartan and candesartan applied in this study are lower than the steady-state plasma concentrations seen in humans with the typical dosage regimens (Marino et al., 1998), they may contribute beneficially to their therapeutic profile.

5. Conclusions

The facilitating effect of angiotensin II on electrical field stimulation-induced sympathetic transmission is mediated by prejunctional angiotensin AT_1 receptors. The order of potency concerning sympatho-inhibition is candesartan> eprosartan. This order of potency also holds for postjunctional inhibition of responses to angiotensin II. Additionally, the sympatho-inhibitory and vascular inhibitory potencies for candesartan appear similar, whereas for eprosartan, a 10-fold difference was observed. The results may be explained by differences between the angiotensin AT_1 receptor subtype on vascular smooth muscle cells and that on the presynaptic membranes of sympathetic varicosities.

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