\textbf{\textit{$\beta_2$-Adrenergic Receptor and Angiotensin II Receptor Modulation of Sympathetic Neurotransmission in Human Atria}}

L.C. Rump, E. Schwertfeger, U. Schaible, G. Fraedrich, P. Schollmeyer

\textbf{Abstract} The aim of the present study was to investigate $\beta$-adrenergic receptor and angiotensin II (Ang II) receptor modulation of norepinephrine release in human atria. Slices of human atrial appendages were incubated with [\textsuperscript{3}H]norepinephrine, superfused with Krebs-Henseleit solution, and electrically stimulated in superfusion chambers. Pretreatment of the tissue with 6-hydroxydopamine (1.2 mmol/L) before the [\textsuperscript{3}H]norepinephrine incubation to destroy sympathetic nerves reduced the uptake of radioactivity and abolished the stimulation-induced (S-I) outflow of radioactivity. Furthermore, S-I outflow of radioactivity was prevented by the addition of tetrodotoxin (1 \textmu mol/L) to and omission of extracellular Ca\textsuperscript{2+} from the superfusion solution. Separation of [\textsuperscript{3}H]norepinephrine from its metabolites revealed that the S-I outflow of radioactivity was mainly composed of intact [\textsuperscript{3}H]norepinephrine. Thus, the S-I outflow of radioactivity was taken as an index of norepinephrine release. Isoproterenol (0.001 to 0.1 \textmu mol/L) dose-dependently enhanced the S-I outflow of radioactivity. The concentration-response curve of isoproterenol was shifted to the right by the selective $\beta_2$-adrenergic receptor antagonist ICI 118551 (0.01 and 0.1 \textmu mol/L) but not by the $\beta_1$-adrenergic receptor-selective antagonist atenolol (0.3 and 30 \textmu mol/L). Ang II (0.001 to 1.0 \textmu mol/L) also dose-dependently enhanced S-I outflow of radioactivity. The facilitatory effect of Ang II was blocked by either the peptide Ang II receptor antagonist saralasin (1.0 \textmu mol/L) or EXP 3174 (0.1 \textmu mol/L), the in vitro active form of the nonpeptide Ang II receptor antagonist losartan. The cell-permeable cAMP analogue 8-bromo-cAMP (30 to 300 \textmu mol/L) dose-dependently enhanced S-I outflow of radioactivity. A combination of a saturating concentration of 8-bromo-cAMP (300 \textmu mol/L), the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX, 90 \textmu mol/L), and isoproterenol (0.1 \textmu mol/L) did not enhance S-I outflow of radioactivity to a greater extent than did isoproterenol (0.1 \textmu mol/L) alone. In contrast, a combination of 8-bromo-cAMP (300 \textmu mol/L), IBMX (90 \textmu mol/L), and Ang II (0.1 \textmu mol/L) enhanced S-I outflow of radioactivity to a greater extent than did Ang II (0.1 \textmu mol/L) alone. The data suggest that norepinephrine release from sympathetic nerves in human atria is facilitated through prejunctional $\beta_2$-adrenergic receptors and Ang II receptors. Prejunctional $\beta_2$-adrenergic receptors but not Ang II receptors seem to be linked to activation of an adenylate cyclase pathway. (Circ Res. 1994;74:434-440.)

Key Words $\cdot$ $\beta_2$-adrenergic receptors $\cdot$ angiotensin II receptors $\cdot$ norepinephrine $\cdot$ human atria

Sympathetic nerve terminals of various tissues possess prejunctionally located receptor systems that, when activated, enhance or inhibit the amount of norepinephrine released per nerve impulse.\textsuperscript{1,4} One of these prejunctional facilitatory receptor systems is mediated via $\beta_2$-adrenergic receptors.\textsuperscript{5,6} $\beta_2$-Adrenergic receptors can be divided in $\beta_1$ and $\beta_2$ subtypes,\textsuperscript{7} and it is generally believed that prejunctional $\beta_2$-adrenergic receptors are of the $\beta_2$ subtype. Most of the studies regarding prejunctional $\beta_2$-adrenergic receptor function have been performed in isolated sympathetically innervated tissues of various animal species.\textsuperscript{5,7} Regarding cardiac sympathetic nerves, it has been shown that activation of prejunctional $\beta_2$-adrenergic receptors facilitates norepinephrine release in isolated atria of guinea pigs,\textsuperscript{8,9} rats,\textsuperscript{10} and mice.\textsuperscript{11} There is now a substantial body of evidence that the human heart possesses both $\beta_1$- and $\beta_2$-adrenergic receptors; however, most functional studies have focused on the role of $\beta_2$-adrenergic receptors on myocardial\textsuperscript{12,13} and sinoatrial\textsuperscript{14} pacemaker cells. Little systematic work has been done regarding prejunctional receptor function in the human heart.\textsuperscript{15,16} Another prejunctional facilitatory system is mediated by angiotensin II (Ang II) receptors.\textsuperscript{1,4,17} Accordingly, it has been shown that Ang II enhances norepinephrine release in guinea pig,\textsuperscript{18} mouse,\textsuperscript{19} and rat\textsuperscript{20} isolated atria, but no data are available regarding a direct prejunctional effect of Ang II in human cardiac tissue. Therefore, the aim of the present study was (1) to establish a reliable technique that permits investigation of the modulation of norepinephrine release from sympathetic nerves in human atria and (2) to characterize the modulatory effects of $\beta_2$-adrenergic receptor and Ang II receptor activation.

Materials and Methods

Human Right Atrium

The present in vitro study in human right atrial appendages was approved by the local ethics committee. The majority of right atrial appendages was taken from 57 patients (33 to 77 years of age; mean age, 60.3 \pm 1.2 years) undergoing open heart surgery for coronary bypass grafting with no signs of heart failure. Four patients were classified according to the New York Heart Association (NYHA) as NYHA II to III. None of the patients had been treated with drugs known to interact with either the storage or release mechanism of norepineph-
rne. Four patients had received β-adrenergic receptor-blocking drugs, and three patients had received angiotensin-converting enzyme inhibitors. The right atrial appendages were cleared from all connective tissue, and slices (≈0.3 mm thick; diameter, 6.0 mm) were prepared.

**Experimental Design**

The slices were incubated with (−)-[2,5,6,3H]norepinephrine (S2, 1 Ci/mmol, 0.5 μmol/L) for 60 minutes in Krebs-Henseleit solution that was continuously bubbled with carbogen (95% O2–5% CO2). The slices were then placed into six superfusion chambers (volume, 250 μL) between two platinum electrodes and were kept in a bath at a temperature of 37°C as previously described for human renal cortex.21 The slices were superfused at a constant rate of 2.5 mL/min with Krebs-Henseleit solution for 141 minutes to remove loosely bound radioactivity. After 120 minutes of this washing procedure, a priming stimulation (10 Hz, 38-mA current strength, 1-milliampere) was added to the superfusion solution. Then, after the washing, consecutive supernates of the superfusate were collected in 3-minute fractions with a fraction collector (Isco Retriever IV, Isco, Lincoln, Neb). There were four stimulation periods (S1 to S4, 27 minutes apart, 38 mA, 10 Hz for 1 minute). A few experiments were performed with only two electrical stimulations (S1 and S2). S1 always served as a reference stimulation. The effect of drugs was tested by adding them in increasing concentrations to the superfusion solution 12 minutes before S1, S2, and S3. In those experiments in which a drug was present for both stimulation periods (throughout), it was added to the superfusion solution immediately after the priming stimulation. In some experiments, the superfusate samples were tested for their [3H]norepinephrine content by sequential alumina and Dowex column chromatography.22

**Estimation of Radioactivity**

The collected 3-minute samples of the superfusion solution were mixed with 8 mL scintillation fluid (Picofluor 40, Packard Instruments, Downers Grove, Ill) to measure the amount of radioactivity present in the superfusion solution by liquid scintillation counting. Total tissue radioactivity was determined at the end of each experiment. The atrial slices were dissolved in 1 mL tissue solubilizer (Solune, Packard Instruments) and then mixed with 8 mL scintillation fluid.

**Calculation of Results**

The spontaneous outflow of radioactivity from the slices was determined as the mean of the amount of radioactivity in the superfusate collected during the 3-minute collection period immediately before and 12 minutes after the onset of stimulations (S1 to S4). The stimulation-induced (S-I) outflow of radioactivity was calculated by subtracting the spontaneous outflow of radioactivity from the radioactivity present in the four 3-minute samples collected immediately after the onset of stimulation. The S-I outflow of radioactivity is subsequently expressed as a fraction (percentage) of the total tissue content of radioactivity at the time of stimulation (fractional S-I outflow of radioactivity [FR]). S2 (FR2) served as a reference stimulation, and the fractional S-I outflow of radioactivity in S1 (FR1 to FR2) is expressed as a percentage of that in S2 (FR2, as a percentage of FR2). The spontaneous (resting) outflow of radioactivity during S1 is expressed as a percentage of that during S2 (R1, as a percentage of R2). For further evaluation of drug effects, the FR1/FR2 values were calculated as a percentage of the values in the corresponding control experiments. The pKb value for ICI 118551 was calculated according to Equation 4 of Furchgott.23 All data are expressed as mean±SEM. Multiple slices were prepared from each atrium, so that different treatments could be tested in one atrium; n gives the number of atria used in each group. Data were analyzed by a modified t test according to Bonferroni or ANOVA where appropriate. Values of P<.05 were considered statistically significant.

**6-Hydroxydopamine Pretreatment**

Some experiments were performed in atrial slices that were pretreated with 6-hydroxydopamine (1.2 mmol/L) for 10 minutes before incubation with [3H]norepinephrine as previously described for rat caudal artery and portal vein24 and human kidney cortex.21 In this case, NaHCO3 and KH2PO4 were omitted from the Krebs-Henseleit solution. Glutathione was added to give a final concentration of 20 μmol/L. The pH of this solution was between 3.0 and 4.0. Vehicle control experiments were always run in parallel.

**Determination of Tissue Levels of Endogenous Norepinephrine**

Right atrial appendages were weighed and immediately placed into centrifugation tubes containing perchloric acid (0.1 μmol/L). The atrial appendages were homogenized and then centrifuged at 10 000 rpm for 15 minutes at 4°C. The endogenous norepinephrine levels were measured in aliquots of the supernatant by high-pressure liquid chromatography coupled with electrochemical detection as described previously.25

**Frequency-Response Curve of the S-I Outflow of Radioactivity**

In some experiments, the influence of the stimulation frequency on the S-I outflow of radioactivity from human right atrial appendages preincubated with [3H]norepinephrine was tested. After the washing procedure, there were nine stimulation periods each for 1 minute, 38-mA current strength, 1-millisecond pulse width: S1 (1.25 Hz), S2 (2.5 Hz), S3 (5 Hz), S4 (10 Hz), S5 (12.5 Hz), S6 (20 Hz), S7 (25 Hz), S8 (35 Hz), and S9 (50 Hz). The S-I outflow of radioactivity for each stimulation period was calculated as described above (calculation of data).

**Drugs and Vehicles**

The Krebs-Henseleit solution had the following composition (mmol/L): NaCl 118, KCl 4.7, CaCl2 2.5, MgSO4 0.45, NaHCO3 25, KH2PO4 1.03, d(-)-glucose 11.1, disodium edetate 0.067, corticosterone 0.03, and ascorbic acid 0.07.

The following drugs were purchased: levo-[ring-2,5,6-3H]norepinephrine (NEN, Dreieich, Germany); 8-bromocAMP, (±)-isoproterenol hydrochloride, tetrodotoxin, corticosterone, 6-hydroxydopamine hydrobromide, tetrodotoxin, 3-isobutyl-1-methylxanthine (IBMX), and [Sar1,Val2,Ala3]-Ang II (saralasin) (Sigma Chemical Co, St Louis, Mo); and cocaine hydrochloride (Merck, Darmstadt, Germany).

The following drugs were generously donated: erythro-DL-1-(7-methyl-indan-4-ylxylo)-3-isopropylaminobutan-2-ol (ICI 118551) and atenolol (ICI, Macclesfield, UK); [Val5,Ang II (CIBA-GEIGY, Wehr, Germany); and 2-n-buty1-4-chloro-1-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid (EXP 3174, Du Pont, Wilmington, Del). All drugs were dissolved in distilled water except corticosterone and EXP 3174. Corticosterone and EXP 3174 were dissolved in absolute ethanol before being diluted with Krebs-Henseleit solution.

**Results**

Two hundred ten human right atrial appendage slices (mean wet weight, 9.7±0.3 mg) from 57 patients were incubated with [3H]norepinephrine and superfused with Krebs-Henseleit solution in superfusion chambers between two platinum electrodes. The slices accumulated radioactivity (228 000±10 600 dpm/mg). Tissue levels of endogenous norepinephrine were 2.4±0.38 μg/g (n=9).

There were either two or four electrical field stimulations (S1 to S4) at 10 Hz for 1 minute, and the fractional
Fractional Stimulation-Induced Outflow of Radioactivity as a Percentage of the Total Tissue Radioactivity at the Time of Stimulation in the First Stimulation Period in Human Atrial Appendage Slices Preincubated With [3H]Norepinephrine

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>FR\textsubscript{1}, %</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>0.97±0.14</td>
<td>15</td>
</tr>
<tr>
<td>ICI 118551</td>
<td>0.77±0.11</td>
<td>15</td>
</tr>
<tr>
<td>0.1 μmol/L</td>
<td>1.03±0.13</td>
<td>12</td>
</tr>
<tr>
<td>Atenolol</td>
<td>0.3 μmol/L</td>
<td>0.68±0.10</td>
</tr>
<tr>
<td></td>
<td>0.59±0.06</td>
<td>12</td>
</tr>
<tr>
<td>Saralasin, 1 μmol/L</td>
<td>0.9±0.11</td>
<td>10</td>
</tr>
<tr>
<td>EXP 3174, 0.1 μmol/L</td>
<td>0.67±0.09</td>
<td>12</td>
</tr>
</tbody>
</table>

FR\textsubscript{1} indicates fractional stimulation-induced outflow of radioactivity. Values are mean±SEM.

There were four electrical stimulations, 27 minutes apart, at 10 Hz for 1 minute. There were no significant differences between no drug and drug treatments (P>0.05 by modified t test according to Bonferroni).

S-I outflow of radioactivity in S\textsubscript{i} in the absence and presence of drugs is shown in the Table. In some experiments, the superfusate samples were assayed for their [3H]norepinephrine content; unmetabolized [3H]norepinephrine accounted for 82.6±3.1% (n=4) of the S-I outflow of radioactivity. In contrast, the spontaneous (resting) outflow of radioactivity contained only 2.9±0.2% (n=4) unmetabolized [3H]norepinephrine.

Effects of Tetrodotoxin, Omission of Ca\textsuperscript{2+}, and 6-Hydroxydopamine on S-I Outflow of Radioactivity

In control experiments, S-I outflow of radioactivity was constant over four (S\textsubscript{1} to S\textsubscript{4}) stimulation periods (Fig 1). The sodium channel blocker tetrodotoxin (1 μmol/L), which was added to the superfusion solution 12 minutes before S\textsubscript{2}, and the omission of Ca\textsuperscript{2+} abolished S-I outflow of radioactivity (not shown). In vitro pretreatment of the tissue with 6-hydroxydopamine (1.2 mmol/L) before incubation with [3H]norepinephrine reduced total tissue radioactivity and abolished S-I outflow of radioactivity (Fig 1).

Influence of the Stimulation Frequency on the S-I Outflow of Radioactivity

Electrical stimulation from 1.25 to 50 Hz induced a frequency-dependent increase of the S-I outflow of radioactivity (Fig 2). The maximum increase of the S-I outflow of radioactivity was reached at a frequency of 20 Hz.

Fig 1. Graphs showing the effect of 6-hydroxydopamine (6-OH-DA) pretreatment on accumulation of tissue radioactivity (right) and stimulation-induced (S-I) outflow of radioactivity (left) in human atrial appendage slices preincubated with [3H]norepinephrine. Before incubation with [3H]norepinephrine, the slices were either pretreated with 6-OH-DA (1.2 mmol/L, n=4) or vehicle (n=4) for 10 minutes. After incubation with [3H]norepinephrine, the slices were superfused with Krebs-Henseleit solution in superfusion chambers. Consecutive 3-minute fractions of the superfusate were collected. There were four stimulation periods (S\textsubscript{1} to S\textsubscript{4}), each 27 minutes apart at 10 Hz for 60 seconds. Total tissue radioactivity was measured at the end of each experiment. The S-I outflow of radioactivity (S\textsubscript{1} to S\textsubscript{4}) and the basal outflow of radioactivity are expressed as a fraction (percentage) of the total tissue radioactivity at the time of the collection (fractional S-I outflow). All data are mean±SEM.

Fig 2. Frequency-response curve of the fractional stimulation-induced (S-I) outflow of radioactivity as a percentage of total tissue radioactivity at the time of stimulation from human atrial appendage slices preincubated with [3H]norepinephrine. The slices were stimulated with increasing frequencies from 1.25 to 50 Hz, each for 60 seconds (n=7). All data are mean±SEM.
Effect of Isoproterenol on the S-I Outflow of Radioactivity

The β₁- and β₂-adrenergic receptor agonist isoproterenol (0.001 to 0.1 μmol/L) dose-dependently enhanced S-I outflow of radioactivity (Fig 3). The concentration-response curve of isoproterenol was shifted to the right by the β₂-adrenergic receptor-blocking drug ICI 118551 (0.01 μmol/L) with an estimated pKᵦ of 9.1 (Fig 3A). In the presence of ICI 118551 (0.1 μmol/L), the facilitatory effect of isoproterenol (0.001 to 0.1 μmol/L) was totally abolished (Fig 3A). However, the β₁-adrenergic receptor-blocking drug atenolol (0.3 and 30 μmol/L) failed to alter the facilitatory effect of isoproterenol (0.001 to 0.1 μmol/L) (Fig 3B). Isoproterenol (0.01 μmol/L) slightly increased the spontaneous outflow of radioactivity to 78.5±0.7% (n=10) as compared with the control level of 75.4±0.9% (n=15), and isoproterenol (0.1 μmol/L) slightly increased the spontaneous outflow of radioactivity to 74.8±1.2% (n=10) as compared with the control level of 69.6±1.2% (n=15).

Effect of Ang II on the S-I Outflow of Radioactivity

Ang II (0.001 to 0.1 μmol/L) dose-dependently enhanced the S-I outflow of radioactivity (Fig 4). The facilitatory effect of Ang II was markedly attenuated by the Ang II receptor antagonist saralasin (1 μmol/L) and totally abolished by EXP 3174 (0.1 μmol/L) (Fig 4). Ang II (0.01 μmol/L) slightly increased the spontaneous outflow of radioactivity to 89.4±1.8% (n=6) as compared with the control level of 86.0±0.7% (n=12), and Ang II (0.1 μmol/L) slightly increased the spontaneous outflow of radioactivity to 89.8±1.6% (n=6) as compared with the control level of 87.0±0.7% (n=12).
outflow of radioactivity to 77.9±1.2% (n=6) as compared with the control level of 74.7±1.0% (n=12).

**Effect of ICI 118551, Atenolol, Saralasin, and EXP 3174 on the S-I Outflow of Radioactivity**

The effect of the antagonists by themselves was tested in experiments with only two stimulations (S1 and S2), and the antagonists were always added 12 minutes before S2. As compared with the control level (90.8±2.9%, n=10), none of the following antagonists had a significant effect on the S-I outflow of radioactivity: ICI 118551 (0.1 μmol/L, 85.6±4.8%, n=4), atenolol (0.3 μmol/L, 100.5±3.5%, n=5), saralasin (1 μmol/L, 94.4±3.5%, n=4), and EXP 3174 (0.1 μmol/L, 95.7±4.7%, n=6). None of the drugs altered the spontaneous outflow of radioactivity (data not shown).

**Effect of 8-Bromo-cAMP and Combinations of 8-Bromo-cAMP With Various Drugs on the S-I Outflow of Radioactivity**

The cell-permeable cAMP analogue 8-bromo-cAMP (30, 100, and 300 μmol/L), when added in increasing concentrations 12 minutes before S1 to S6, dose-dependently enhanced the S-I outflow of radioactivity to 108.4±6.8%, 126.8±7.0%, and 168.3±14.4% of the control value (n=5), respectively. The following results were obtained in experiments with two stimulations (S1 and S2): A combination of the phosphodiesterase inhibitor IBMX (90 μmol/L) and 8-bromo-cAMP (300 μmol/L), which was added 12 minutes before S2, enhanced the S-I outflow of radioactivity to a slightly greater extent than 8-bromo-cAMP (300 μmol/L) alone (Fig 5). A combination of 8-bromo-cAMP (300 μmol/L)/IBMX (90 μmol/L) and isoproterenol (0.1 μmol/L), which were added together to the superfusion solution 12 minutes before S2, did not enhance S-I outflow of radioactivity to a greater extent than did 8-bromo-cAMP (300 μmol/L)/IBMX (90 μmol/L) alone (Fig 5). In contrast, a combination of 8-bromo-cAMP (300 μmol/L)/IBMX (90 μmol/L) and Ang II (0.1 μmol/L) enhanced S-I outflow of radioactivity to a greater extent than did 8-bromo-cAMP (300 μmol/L)/IBMX (90 μmol/L) alone (Fig 5). 8-Bromo-cAMP (30 to 300 μmol/L) and none of the drug combinations had an effect on the spontaneous outflow of radioactivity (data not shown).

**Discussion**

Human right atrial appendages are densely innervated by the sympathetic nervous system26 and seem to contain large amounts of norepinephrine as measured spectrofluorometrically.27 Comparable levels of norepinephrine were measured in the present study by high-pressure liquid chromatography. For comparison, human kidney cortex contains six times less norepinephrine23 than does human right atrium. Slices of human right atrial appendages, when incubated with tritiated norepinephrine, accumulated and stored radioactivity. Moreover, they released radioactivity on electrical stimulation in a frequency-dependent manner. Pretreatment of the atria with 6-hydroxydopamine, which destroys sympathetic nerve endings,28 reduced the ability to accumulate radioactivity and abolished the S-I outflow of radioactivity. As in previous studies in other sympathetically innervated tissues incubated with [3H]norepinephrine, the spontaneous outflow of radioactivity from the atria was composed mainly of 3H metabolites, whereas intact [3H]-norepinephrine accounted almost entirely for the S-I outflow of radioactivity. The S-I outflow of radioactivity was dependent on extracellular calcium and could be blocked by tetrodotoxin. Thus, superfused atrial slices provide a reliable technique to study the modulation of norepinephrine release from human cardiac sympathetic nerves in vitro.

**β-Adrenergic Receptor Activation and Norepinephrine Release**

The nonselective β1- and β2-adrenergic receptor agonist isoproterenol concentration-dependently enhanced S-I outflow of radioactivity. This facilitatory effect was abolished by the selective β2-adrenergic receptor antagonist ICI 118551 (0.1 μmol/L).29 A 10 times lower concentration of ICI 118551 (0.01 μmol/L) shifted the concentration-response curve of isoproterenol to the right with an estimated pKb23 of 9.1. This pKb for ICI 118551 is almost identical to that obtained for ICI 118551 in guinea pig papillary muscle30 and fits with the inhibition constants of ICI 118551 determined for [3H]dopamine binding and adenylate cyclase activity.31 In contrast, the selective β2-adrenergic receptor antagonist atenolol32 at a concentration of 0.3 μmol/L, and even at 30 μmol/L, did not shift the concentration-response
curve of isoproterenol. Taken together, the data suggest that activation of prejunctional β-adrenergic receptors enhances norepinephrine release from sympathetic nerves in human atria. It has been suggested that norepinephrine activates prejunctional β-adrenergic receptors to form a “positive-feedback loop” on its own release in the guinea pig atria. If prejunctional β-adrenergic receptors were activated by endogenous norepinephrine, then β-adrenergic receptor antagonists should inhibit norepinephrine release. However, the possibility of such a positive-feedback loop seems to be unlikely in human atria, since both CCI 118551 and atenolol failed to alter S-I outflow of radioactivity.

**Ang II Receptor Activation and Norepinephrine Release**

Another facilitatory prejunctional mechanism is mediated via Ang II receptors. Accordingly, Ang II dose-dependently enhanced the S-I outflow of radioactivity from human atria, and the maximal facilitatory effect was reached at a concentration of 0.1 μmol/L. Ang II in high concentrations blocks the uptake of norepinephrine in the rabbit heart, and a similar effect may be responsible for the enhanced S-I outflow of radioactivity in the present study. The uptake-blocking effect of Ang II occurs only at Ang II concentrations of 10 μmol/L, far higher than used in the present study. Moreover, in the present experiments, extraneuronal and neuronal uptake of norepinephrine was already blocked by corticosterone and cocaine. Furthermore, the facilitatory effect of Ang II was inhibited by the peptide Ang II receptor antagonist saralasin and EXP 3174, which in vitro active form of the recently developed nonpeptide Ang II receptor antagonist losartan. Thus, the facilitatory effect of Ang II seems to be due to activation of prejunctional Ang II receptors. An inhibitory effect of losartan on prejunctional Ang II receptor–mediated facilitation of transmitter release has been previously reported for isolated artery preparations. Saralasin is a peptide Ang II receptor antagonist with partial agonist activity at Ang II receptors. However, saralasin (1 μmol/L) by itself did not enhance S-I outflow of radioactivity from human atrial slices preincubated with [3H]norepinephrine. This is in contrast to observations in the rat isolated kidney. EXP 3174 by itself also failed to alter the S-I outflow of radioactivity, which suggests that under the experimental conditions used endogenous Ang II does not activate prejunctional Ang II receptors to enhance norepinephrine release in superfused human atrial slices.

There are only a few and controversial reports about prejunctional facilitatory effects of Ang II in humans. In an early study by Mendelsohn et al., infusion of Ang II into resting normotensive subjects failed to increase norepinephrine plasma levels. However, a more recent study in healthy normotensive volunteers has demonstrated a prejunctional facilitatory effect of Ang II on sympathetic neurotransmission. In this particular study, the effect of Ang II infused into the left brachial artery on the reduction in forearm blood flow induced by lower-body negative pressure was examined. Ang II by itself did not significantly alter forearm blood flow but enhanced the vasoconstrictor response to lower-body negative pressure without affecting the response to exogenous norepinephrine. Thus, it appears that only in situations involving an activated sympathetic nervous system (lower-body negative pressure) does a prejunctional effect of Ang II become detectable. Heart failure is a clinical condition with an activated sympathetic nervous and renin-angiotensin system. Therefore, one could speculate that beneficial effects of Ang II receptor–blocking drugs and ACE inhibitors in the treatment of heart failure may be partly due to the prevention of facilitatory effects of endogenous Ang II on sympathetic neurotransmission.

**cAMP and Norepinephrine Release**

The cell-permeable stable cAMP analogue 8-bromo-cAMP dose-dependently enhanced S-I outflow of radioactivity from human atria preincubated with [3H]norepinephrine but had no effect on the spontaneous outflow. This is in accord with findings of Cibello et al., who determined that cAMP is only involved in the nerve stimulation–induced release of norepinephrine but does not interact with the norepinephrine release process itself. A dose-dependent facilitatory effect of cell-permeable cAMP analogues has been previously observed in mouse atria and the human pulmonary artery. It has been suggested that the facilitatory effect of Ang II receptor activation on norepinephrine release is linked to stimulation of adenylate cyclase and, hence, an increased formation of cAMP. In the present study, a combination of 8-bromo-cAMP and the nonspecific phosphodiesterase inhibitor IBMX was used to test this hypothesis. If intraneuronal levels of 8-bromo-cAMP and endogenous cAMP were already maximally increased, then isoproterenol should not be able to enhance neurotransmitter release through a cAMP-dependent mechanism. And indeed, when the maximal facilitatory concentration of isoproterenol (0.1 μmol/L) and the combination 8-bromo-cAMP (300 μmol/L)/IBMX (90 μmol/L) were added together, their facilitatory effects were not additive. This seems not to be an unspecific effect of 8-bromo-cAMP/IBMX on neurotransmitter release, since Ang II (0.1 μmol/L) and the combination 8-bromo-cAMP/IBMX enhanced the S-I outflow of radioactivity to a greater extent than did Ang II alone. Our data indicate that β-adrenergic receptor–in contrast to Ang II receptor–mediated facilitation of norepinephrine release in human atria involves cAMP as a second messenger. Similar conclusions have been drawn in previous studies involving mouse atria, rabbit pulmonary artery, and rat renal cortex.

In conclusion, the amount of norepinephrine released per nerve impulse from sympathetic nerves in human atria is facilitated through activation of prejunctional β-adrenergic receptors and Ang II receptors. The cell-permeable stable cAMP analogue 8-bromo-cAMP enhances norepinephrine release from human atria, and β-adrenergic receptor but not Ang II receptor facilitation of norepinephrine release seems to be linked to an adenylate cyclase pathway.

**Acknowledgment**

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