Isoproterenol-Induced Facilitation of Norepinephrine Release Does Not Primarily Involve a Local Angiotensin II Mechanism in Guinea Pig Pulmonary Arteries

Misako KUWAHARA, Takao KUBO and Yoshimi MISU
Department of Pharmacology, Yokohama City University School of Medicine, Yokohama 236, Japan
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Abstract—We have attempted to clarify whether or not captopril and 1Sar-8Ile-angiotensin II could protect isoproterenol-induced facilitation of norepinephrine release in guinea pig pulmonary arteries loaded with 3H-norepinephrine. Angiotensin I at 1 nM and 30 nM isoproterenol similarly facilitated evoked release of 3H-norepinephrine at 1 Hz. Captopril at 1 μM and 1Sar-8Ile-angiotensin II at 10 nM completely prevented angiotensin I-induced facilitation, whereas these pretreatments produced no effect on isoproterenol-induced facilitation. Isoproterenol-induced facilitation of norepinephrine release does not primarily involve a local angiotensin II mechanism.

Presynaptic β-adrenoceptors have been thought to mediate a positive feedback mechanism for the release of norepinephrine (1). Recently, this positive feedback mechanism in some vascular tissues has been suggested at least in part to be due to β-agonist-stimulated local angiotension II formation and subsequent activation of presynaptic angiotensin II receptors (2–4). However, contrary evidence has also been shown in mouse atria (5) and rat kidney (6). We already established the existence of presynaptic β-adrenoceptors (7–10) and presynaptic angiotensin II receptors (11) in guinea pig pulmonary arteries. We have attempted to clarify whether or not this angiotensin II-mediated mechanism is primarily the case in these arteries.

Spirally cut preparations of the pulmonary arteries from male guinea pig weighing 200 to 250 g were prepared and incubated at 37°C for 60 min with oxygenated Krebs bicarbonate solution containing 0.1 μM 3H-norepinephrine (Amersham/Searle) and 100 mg/l ascorbic acid, and then they were mounted vertically between a pair of platinum stimulating electrodes and superfused with Krebs medium at a constant rate of 0.5 ml/min as described previously (7–10). The solution, maintained at 37°C, pH 7.2 to 7.4, was bubbled with 5% CO2 in O2. A 90-min equilibration period was allowed, and then transmural field stimulations with electrical rectangular pulses (2 msec, 10 V, 1 Hz and 100 sec) were repeated twice (S1 and S2 periods) at a 30 min interval, using an electrical stimulator with an isolator (SEN-3201 and SS-120J, Nihon Kohden). Superfusate for 2 min was collected before, during and after the field stimulation, respectively; then 12 ml ACS-II solution was added to each sample, and total 3H-activities expressed as disintegration per min were determined using a liquid scintillation spectrometer (Beckman LS 5800). The impulse-evoked release (S) of 3H was calculated as the difference between resting efflux before stimulation and total efflux detected in 3 successive samples during and after stimulation. The resting efflux was regarded as the spontaneous release (Sp). Angiotensin I (Sankyo) at 1 nM or /-isoproterenol hydrochloride (Sigma) at 30 nM was applied 20 min before the S2 stimulation. Captopril (Sankyo), a converting enzyme inhibitor, at 1 μM and 1Sar-8Ile-angiotensin II (Daichi Seiyaku), an angiotensin II antagonist, at 10 nM was applied 30 min before
the $S_1$ period. The effect of angiotensin I and isoproterenol was evaluated by the $%$ release ratios of $S_2/S_1$ and $Sp_2/Sp_1$. All of these experiments were done in the absence of drugs such as cocaine or $\alpha_2$-adrenoceptor antagonists. Drugs used were dissolved in distilled water before use. Data shown are means±S.E., and statistical significance was calculated using the unpaired Wilcoxon's rank-sum test.

In control preparations, the spontaneous release and the release evoked at 1 Hz for 100 sec from spirally cut guinea pig pulmonary arteries loaded with $^3$H-norepinephrine was 2145.1±80.4 dpm/tissue/2 min and 5973.4±696.6 dpm/tissue ($n=6$), respectively, 90 min after the start of superfusion. Isoproterenol at 30 nM facilitated the evoked release of $^3$H-norepinephrine (Table 1). This 30 nM is approximately the 50% effective concentration from the concentration-release-curve for 1 nM to 1 $\mu$M isoproterenol (9). This isoproterenol-induced facilitation has been interpreted as that due to the activation of tonically functioning presynaptic $\beta_2$-adrenoceptors (1, 7–10): for example, the facilitation of the norepinephrine release by 0.1 $\mu$M isoproterenol was completely and stereoselectively antagonized by 0.1 $\mu$M propranolol, and this concentration of the antagonist alone stereoselectively inhibited the contractile responses of the radial muscle preparations of these arteries to sympathetic nerve stimulation without antagonizing contractile responses to exogenously applied norepinephrine (7).

On the other hand, this isoproterenol-induced facilitation also has been suggested at least partially in some vascular tissues to be due to the local angiotensin II formation via the activation of non-neuronal $\beta_2$-adrenoceptors and subsequent activation of presynaptic angiotensin II receptors: 0.1 nM to 1 $\mu$M isoproterenol concentration-dependently facilitated the noradrenergic neurotransmission in perfused mesenteric beds (2, 3) and in vena cava (4) from rats. In general, the concentrations of drugs used in these experiments (2–4) seem to be relatively high. The concentration-response-curves for isoproterenol at high concentrations of 50 nM to 1 $\mu$M were partially suppressed by the moderate concentrations of captopril (0.5 and 1 $\mu$M) and also by a relatively high concentration saralasin (0.1 $\mu$M), an angiotensin II antagonist. Moreover, these curves were almost completely suppressed by the relatively high concentrations of 2 and 5 $\mu$M captopril and by the high concentrations of 0.5 and 1

| Pretreatments (nM) | Agonists (nM) | N  | $S_2/S_1$ (%) | $Sp_2/Sp_1$ (%) |
|-------------------|--------------|----|---------------|----------------|
| None              | None         | 6  | 93.5±4.8      | 83.6±2.6       |
| None              | Ang. I 1     | 6  | 161.6±8.9*    | 78.7±1.9       |
| Captopril 1000    | Ang. I 1     | 4  | 91.6±4.3†     | 81.3±1.5       |
| 1Sar-8Ile-Ang. II 10 | Ang. I 1     | 4  | 94.3±6.1†     | 84.4±2.7       |
| None              | Isoproterenol 30 | 12 | 142.9±4.4*    | 86.0±1.8       |
| Captopril 1000    | Isoproterenol 30 | 8  | 148.3±10.1*   | 84.8±4.6       |
| 1Sar-8Ile-Ang. II 10 | Isoproterenol 30 | 8  | 128.4±5.3*    | 82.7±2.3       |

Strips were incubated for 60 min in 0.1 $\mu$M $^3$H-norepinephrine, rinsed, set up, and then superfused with Krebs solution. Transmural field stimulation (1 Hz, 2 msec, 10 V, 100 pulses) was done twice 90 min ($S_1$) and 120 min ($S_2$) after the start of superfusion. Angiotensin (Ang.) I and isoproterenol was applied 20 min before $S_2$, and captopril and 1Sar-8Ile-Ang. II was applied 30 min before $S_1$. Effects of the agonists on the evoked release ($S$) and spontaneous release ($Sp$) are expressed as $%$ release ratios, $S_2/S_1$ and $Sp_2/Sp_1$. Data shown are means±S.E., and N is number of estimations. Statistical significance: *$P<0.01$, compared to the control; †$P<0.01$, compared to Ang. I alone.
μM 1Sar-8Ile-angiotensin II (2–4). Isoproterenol at 10 nM to 1 μM concentration-dependently increased the overflow of angiotensin II, and the effect of 1 μM isoproterenol was approximately half antagonized by 1 μM propranolol (3). Another important fact is that when captopril could suppress the concentration-response-curves for isoproterenol, the effect of captopril alone on noradrenergic neurotransmission should be carefully determined, since captopril attenuated the release of norepinephrine in guinea pig pulmonary arteries (11) and rat perfused mesenteric beds (12).

In guinea pig pulmonary arteries (11), approximately one quarter of the exogenously applied angiotensin I was converted to angiotensin II, which subsequently facilitated the impulse-evoked release of norepinephrine via presynaptic angiotensin II receptors. As shown in Table 1, 1 nM angiotensin I facilitated the release of norepinephrine to a similar degree to that elicited by 30 nM isoproterenol, and this facilitation was completely prevented by captopril at the moderate concentration of captopril. Moreover, the low concentration of 1Sar-8Ile-angiotensin II, 10 nM, also completely antagonized the angiotensin I-induced facilitation of the release of norepinephrine. These results are consistent with our previous findings (11). An important finding in the present experiments is that the pretreatment with this carefully selected respective concentration of captopril and 1Sar-8Ile-angiotensin II produced no effect on the isoproterenol (30 nM)-induced facilitation of the norepinephrine release (Table 1). The control Sp2/Sp1 ratio was not modified by each procedure done in the present experiments. It was confirmed that the pretreatment with 1 μM captopril by itself produced no modifications of the S2/S1 ratio (95.9±6.1%, n=4) and the Sp2/Sp1 ratio (84.8±3.8%). 1Sar-8Ile-angiotensin II alone produced no effect on the norepinephrine release (11).

From the present findings, a primary action of isoproterenol for the facilitation of noradrenergic neurotransmission appears to be the activation of presynaptic β-adrenoceptors. Furthermore, postsynaptic structures are not involved in the activation of presynaptic β-adrenoceptors, since this activation is seen in axonal sprouts from cultured rat superior cervical ganglia (13). In conclusion, the isoproterenol-induced facilitation of the norepinephrine release does not primarily involve a local angiotensin II mechanism in guinea pig pulmonary arteries.

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