Heterogeneity in Vasorelaxant Effects of α-Human Atrial Natriuretic Polypeptide in the Dog

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Abstract—Effects of α-human natriuretic polypeptide (ANP) on isolated canine vascular strips were examined in 6 arteries and 5 veins. Under the preconstriction with either methoxamine, prostaglandin F$_{2\alpha}$ or KCl, ANP relaxed the vascular strips to various degrees, depending on the region removed from the circulatory system. The largest relaxation was obtained in the pulmonary artery, suggesting that the pulmonary artery may be an important site of action of ANP.

ANP is known to play an important role in the regulation of blood pressure as well as in the fluid and electrolyte balance (1, 2). It has been reported that the vasodilating effects of ANP varies considerably among different blood vessels and/or between species. In the rat, ANP induced a vasodilatation in the aorta and renal artery, whereas in the mesenteric arterial perfusion preparation and isolated strips, no relaxant effects of ANP were seen (3). Vasorelaxant effects of ANP has also been investigated in the rabbit arteries and veins systematically (4). Several arteries such as the aorta, renal, mesenteric and carotid arteries were effectively relaxed by ANP, whereas more peripheral segments of the arterial tree such as the saphenous, basilar and ear arteries and large veins were poorly responsive to ANP. In the present study, we examined the vasorelaxant effects of ANP on the isolated dog arteries and veins, systematically.

Dogs weighing 6–12 kg were anesthetized with intravenous injection of pentobarbital sodium (35 mg/kg). Segments of arteries and veins were removed from 11 sites in the circulatory system and were immediately placed in Krebs-Henseleit solution aerated with 95% O$_2$ and 5% CO$_2$. The composition of the solution was as follows: 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO$_4$, 2.6 mM CaCl$_2$, 1.18 mM KH$_2$PO$_4$, 24.9 mM NaHCO$_3$, and 10 mM glucose; the pH of the solution was 7.4 and the temperature was kept at 37°C. Arteries and veins examined in this study were as follows: pulmonary, renal, mesenteric, basilar, coronary, and femoral arteries and pulmonary, renal, mesenteric, jugular, and saphenous veins. Spiral strips were prepared from each vessel except the basilar artery, from which a ring preparation was made. An isometric tension was measured by a force-transducer (Kyowa Dengyo, DPM-110B) and recorded on an ink-writing oscillograph (Watanabe, SR-6202).

Vascular preparations were preconstricted with either methoxamine hydrochloride (Nippon Shinyaku $3\times10^{-6}$ M), prostaglandin F$_{2\alpha}$ (Ono Pharmaceutical Co., Prostarmon) $10^{-5}$ M or KCl (40 mM). The preconstriction levels in each blood vessel ranged from 60% to 90% of the maximal contraction. After a steady level of contraction was achieved, synthetized α-human ANP, supplied from the Suntory Institute of Biochemical Research, was administered at a concentration of 100 ng/ml. Further increases in concentration up
to 1 μg/ml of ANP caused no additional relaxation in all preparations, the responses being estimated to be maximal. The responses were expressed as the percent value of the maximal relaxation caused by the following administration of papaverine hydrochloride (Tokyo Kasei) (10^-4 M). In the pulmonary and renal arteries, ANP was administered cumulatively after the constriction caused by methoxamine (3×10^-5 M) reached a steady level, and concentration-response curves of ANP were obtained.

Relaxations of pulmonary and renal arterial strips to ANP are shown in Fig. 1A. Both strips were relaxed dose-dependently. It was to be noted that the maximal relaxation caused by ANP in the pulmonary artery reached about 70% of the maximal response to papaverine, against 22% in the renal artery. As shown in Fig. 1B, the mean values of relaxation to ANP were significantly larger in the pulmonary artery (n=9) than in the renal artery (n=8) (P<0.05 at 1 and 3 ng/ml of ANP, P<0.01 at 10, 30 and 100 ng/ml, by Student's t-test).

The maximal relaxations caused by ANP in 6 arteries and 5 veins are summarized in Table 1. In each preconstriction of vascular strips with methoxamine, prostaglandin F2α or KCl, the mean values of the maximal responses in the pulmonary artery were the largest among the 11 blood vessels. Each pulmonary arterial strip was relaxed by ANP more than 40% of the maximal response to papaverine under the preconstriction with methoxamine, whereas for other vascular strips, less than 40% under either pretreatment. Under the pretreatment with methoxamine, pulmonary and femoral arterial strips were relaxed by ANP more greatly than with KCl.
Table 1. Maximal relaxations of arterial and venous strips induced by α-human atrial natriuretic polypeptide after preconstriction with methoxamine, prostaglandin F$_{2\alpha}$ and KCl

<table>
<thead>
<tr>
<th>Blood vessels</th>
<th>Methoxamine (%)</th>
<th>PGF$_{2\alpha}$ (%)</th>
<th>KCl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary artery</td>
<td>67.7±8.1 (6)*</td>
<td>85.4±6.7 (5)*</td>
<td>36.8±9.8 (5)</td>
</tr>
<tr>
<td>Renal artery</td>
<td>14.7±5.3 (7)</td>
<td>22.5±3.6 (6)</td>
<td>8.5±7.9 (5)</td>
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<tr>
<td>Mesenteric artery</td>
<td>10.6±3.9 (7)</td>
<td>9.2±6.3 (4)</td>
<td>7.9±5.9 (6)</td>
</tr>
<tr>
<td>Basilar artery</td>
<td></td>
<td>25.6±4.8 (5)</td>
<td>18.2±3.8 (4)</td>
</tr>
<tr>
<td>Coronary artery</td>
<td>16.0±4.9 (8)*</td>
<td></td>
<td>1.3±1.3 (5)</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>13.7±3.4 (8)</td>
<td>10.8±4.4 (7)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary vein</td>
<td>0</td>
<td>3.3±3.3 (4)</td>
<td></td>
</tr>
<tr>
<td>Renal vein</td>
<td>6.3±2.4 (8)</td>
<td>3.3±3.3 (6)</td>
<td></td>
</tr>
<tr>
<td>Mesenteric vein</td>
<td>9.7±3.7 (4)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Jugular vein</td>
<td>6.9±3.1 (6)</td>
<td>1.7±1.2 (4)</td>
<td></td>
</tr>
</tbody>
</table>

Methoxamine (3×10$^{-6}$ M) was used to preconstrict the isolated vascular strips before the administration of α-human atrial natriuretic polypeptide, except in the basilar and coronary arterial strips which were treated with prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$, 10$^{-6}$ M). The concentration of KCl was 40 mM. The maximal relaxation was obtained at 100-300 ng/ml of α-human atrial natriuretic polypeptide. Each value is the mean±S.E. Number in parenthesis indicates the number of experiments. *P<0.05 vs. KCl.

Relaxation to ANP was smaller in vascular strips preconstricted with KCl than with methoxamine or prostaglandin F$_{2\alpha}$, as shown previously (5). This phenomenon was extremely apparent in the pulmonary and femoral arteries (P<0.05). Chiu et al. (6) reported that in the rabbit aorta, the inhibition of contractile responses to norepinephrine and angiotensin II by atriopeptin II was accompanied by significant decreases in 45Ca influx, whereas atriopeptin II exhibited only a modest inhibition on KCl-induced contraction without affecting the accompanying increase in 45Ca influx. Similar findings were reported by Taylor and Meisner (7). As suggested by these authors, ANP may exert its vasorelaxant effect partly via an inhibition of Ca$^{2+}$ influx through receptor-operated Ca$^{2+}$ channels. It was speculated that the regional difference in relaxation to ANP might be due to the difference in ANP-induced inhibitory response to Ca$^{2+}$ influx.

The relaxation of an arterial strip tended to be larger than that of the corresponding venous strip. Such a difference was suggested previously to be independent of the endothelial relaxant factor (4). It has been reported that ANP activates the particulate guanylate cyclase, being different from the nitrovasodilators such as nitroglycerin and nitroprusside, which activate the soluble guanylate cyclase (8) and relax the venous strips effectively (4). However, it is still obscure how the poor ability of ANP to relax the venous strips is related to the activation of particulate guanylate cyclase.

Among the vascular strips examined in this study, the pulmonary arterial strips were relaxed by ANP most greatly in dogs. O'Donnel et al. (9) showed that atriopeptins were potent relaxants in guinea pig tracheal and pulmonary arterial strips, suggesting that atriopeptins played an important role in the regulation of pulmonary vascular and bronchomotor tones. Since the presence of ANP and ANP mRNA in lungs has been demonstrated (10), it is possible that the lungs may play a role in blood pooling under certain conditions, via the potent vasodilatory effect of ANP on the pulmonary artery.

In the rabbit, ANP relaxed the renal artery (>80% of active tension induced by serotonin) more effectively than the pulmonary artery (4). In the dog, however, the relaxant effect of ANP on the renal arterial strip was much smaller than that on the pulmonary arterial strip. Furthermore, the negative logarithm of EC50 of ANP in the renal artery was 7.9±0.1 (n=8), which was significantly smaller than that in the pulmonary artery (8.7±0.2, n=9) (P<0.05). As well known,
i.v. injection of ANP exerts diuresis and natriuresis in the kidney (11). It has been reported that in dogs, a low dose of ANP caused significant diuresis and natriuresis with no change in renal blood flow and glomerular filtration rate (12). So far, such a species difference remains to be elucidated.

References


