Different Modulation by Cyclooxygenase Inhibitors of the Response to Angiotensin II in Monkey Arteries and Veins

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ABSTRACT—In coronary, renal and femoral arteries and mesenteric veins isolated from Japanese monkeys, tachyphylaxis to angiotensin (ANG) II (10^(-7) M)-induced contraction rapidly developed. Contractions caused by ANG II in coronary arteries were attenuated by treatment with indomethacin and aspirin and also by endothelium denudation. Indomethacin inhibited the response of the arteries with and without endothelium to a similar extent. OKY 046, a thromboxane A2 synthesis inhibitor, failed to inhibit the response. In contrast, contractions of renal arteries were potentiated and prolonged by the cyclooxygenase inhibitors. ANG II-induced contractions of mesenteric veins were prolonged but those of femoral arteries were not altered by indomethacin. It is concluded that ANG II contracts monkey coronary arteries, possibly due to the release of vasoconstrictor prostanoids but not thromboxane A2 from endothelial and subendothelial tissues and also due to its direct action on smooth muscle, whereas contractions of renal arteries and mesenteric veins are blunted by vasoconstrictor prostanoids, possibly PGI2. Cyclooxygenase products even if released do not appear to regulate femoral artery contractions produced by ANG II.

Heterogeneity of the response to angiotensin (ANG) II has been determined in a variety of dog arteries (1), cerebral arteries from various mammals (2) and the proximal and distal portions of monkey and dog arteries (3-5). In some blood vessels, such as dog renal and cerebral arteries and mesenteric and pulmonary veins, ANG II produces a relaxation, possibly due to PGI2 release from endothelial and subendothelial tissues (6-8). In the arteries responding to the peptide only with contractions, treatment with cyclooxygenase inhibitors potentiates the contraction (5, 9-12), suggesting that vasodilator prostaglandins (PGs) oppose the vasoconstrictor effect of ANG II on vascular smooth muscle. Tachyphylaxis to ANG II may be related to the release of prostanoids that antagonize ANG II responses (13).

Recently, we have reported that vasoconstrictor PGs released from endothelium are mainly involved in ANG II-induced contractions of dog and monkey cerebral arteries but not monkey mesenteric arteries (14). However, heterogeneity in response to the peptide has not been determined in other monkey blood vessels. The aim of the present study was therefore to evaluate the response to ANG II and the mechanism of its action, with reference to cyclooxygenase products, in...
coronary, renal and femoral arteries and mesenteric veins obtained from Japanese monkeys.

MATERIALS AND METHODS

Preparation

Japanese monkeys of either sex, weighing 6 to 12 kg, were anesthetized with intramuscular injections of ketamine (25 mg/kg) and then killed by bleeding from the carotid arteries. The heart was rapidly removed, and descending and circumflex branches of the left coronary artery were isolated. Intrarenal, interlobar branches of the renal artery were isolated from the kidney. Distal femoral arteries and mesenteric veins were also removed from the monkeys. The arteries and veins were helically cut into strips, approximately 20 mm long. The specimen was vertically fixed between hooks in a muscle bath containing modified Ringer-Locke solution, which was maintained at 37 ± 0.3°C and aerated with a mixture of 95% O₂ and 5% CO₂. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer. The resting tension was adjusted to 1 g for the artery strips and 0.7 g for the vein strips, which are optimal for inducing the maximal contraction. Composition of the solution was as follows: 120 mM NaCl, 5.5 mM KCl, 2.2 mM CaCl₂, 1.0 mM MgCl₂, 25.0 mM NaHCO₃, and 5.6 mM dextrose. The pH of the solution was 7.38 to 7.42. Before the start of the experiments, all of the strips were allowed to equilibrate for 90 to 120 min in the bathing media, during which time the fluid was replaced every 10 to 15 min.

Recording

Isometric contractions and relaxations were displayed on an ink-writing oscillograph (Nihon Kohden Kogyo Co., Tokyo, Japan). The contractile responses to 30 mM K⁺ in the arteries or those to 5 mM Ba⁴⁺ in the veins were first obtained, and the preparations were repeatedly washed and equilibrated. Only a single concentration of ANG II (10⁻⁷ M) that produced maximal contractions in the blood vessels used was applied in each series directly to the bathing media. Preparations had been treated for 20 to 30 min with blocking agents before the response to ANG II was obtained. In some coronary artery strips, the endothelium was removed by gently rubbing the intimal surface with a cotton pellet, and the response of endothelium-denuded strips was compared with that of strips with intact endothelium obtained from the same monkeys. Removal of endothelium was determined by a marked suppression or abolition of relaxation induced by acetylcholine and also histologically ascertained by the method reported by Abrol et al. (15).

Statistics and drugs

Results shown in the text, table and figures are expressed as mean values ± S.E.M. All reported n values refer to the number of monkeys used. Statistical analyses were made using Student’s paired and unpaired t-test. Drugs used were angiotensin (ANG) II, saralasin (¹Sar, ⁸Ala-ANG II; Peptide Institute, Minoh, Japan), acetylcholine chloride (Daiichi Pharmaceutical Co., Tokyo), indomethacin (Sigma, St. Louis, MO), acetyl salicylic acid (aspirin; Nacalai Tesque, Kyoto, Japan) and OKY 046 (E)-3-[4-(1-imidazolylethyl)phenyl]-2 propenoic acid hydrochloride monohydrate (Ono Pharmaceutical Co., Osaka, Japan).

RESULTS

The addition of ANG II in a concentration of 10⁻⁷ M produced a transient contraction in the strips of monkey coronary, renal and femoral arteries and mesenteric veins. In all the vascular strips used, tachyphylaxis developed by repeated applications of the peptide. The responses to the second trial were markedly less than those to the first trial; the responses were usually stabilized after 3 to 5 trials. Therefore, in each strip, ANG II-induced contractions were repeatedly obtained, until the responses were stabilized. After the response in each trial was completed, vascular strips
were washed and equilibrated. Average responses to the first trials and under stabilized conditions in different blood vessels are summarized in Table 1. Femoral arteries and mesenteric veins responded to first application of ANG II with a similar magnitude of contraction to that caused by 30 mM K⁺ or 5 mM Ba⁺⁺, respectively, which were reduced approximately one half during the repeated trials. In coronary and renal arteries, first responses to the peptide were less than those to 30 mM K⁺. Coronary artery contraction in the first trial and under stabilized condition were significantly different in paired comparison (100 vs. 65.8 ± 8.7%, n = 14, P < 0.01). Tachyphylaxis was most evident in the renal arteries.

After the response to ANG II was stabilized, vascular strips were treated for 20 to 30 min with 10⁻⁶ M indomethacin or 5 × 10⁻⁵ M aspirin, which was sufficient to abolish the response mediated via vasodilator PGs released by stimulation of ANG II receptors (16). Contractions caused by the peptide developed more slowly and persisted longer in coronary arteries than in the other arteries and veins (Fig. 1). Treatment with indomethacin or aspirin moderately attenuated the response of coronary arteries; the attenuation was reversed by repeated washing of the strips (Figs. 1 and 2). Endothelium denudation abolished or markedly suppressed the relaxation caused by acetylcholine (up to 10⁻⁵ M), and it significantly decreased the ANG II-induced contraction by 33.7% (Fig. 2). In paired analyses in the arteries with and without endothelium, indomethacin inhibited the contractions by 71.2 ± 10.8 and 72.5 ± 6.6% (n = 6), respectively. Contractions induced by the peptide were abolished by 10⁻⁷ M saralasin in the control and indomethacin-treated arteries (n = 3). Treatment with 10⁻⁵ M OKY 046 did not significantly alter the response to ANG II (16.8 ± 4.3 vs. 18.8 ± 3.3%, n = 4, relative to contractions caused by 30 mM K⁺).

Contractions of renal arteries induced by ANG II (10⁻⁷ M) were potentiated by treatment with indomethacin and aspirin (Figs. 1 and 3). The duration of contraction at the level of half maximal contraction was significantly increased from 4.47 ± 1.10 to 11.6 ± 2.38 min (n = 6, P < 0.05) following treatment with indomethacin. Similar prolongation of the contraction was also obtained with aspirin (n = 3).

Indomethacin and aspirin did not potentiate the response to ANG II of the mesenteric veins (Fig. 3). However, the contraction was significantly prolonged; mean values of the duration at the level of half maximal contrac-

| Table 1. Contractions caused by ANG II (10⁻⁷ M) in the first trial and under the stabilized condition in monkey coronary, renal and femoral arteries and mesenteric veins |
|-----------------------------------------------|
| N | K⁺ * or Ba⁺⁺ ** contraction (mg) | ANG II (first) (%) | ANG II (stabilized) # # |
|-----------------|---------------------|-----------------|-----------------|
| Coronary artery | 15 1341 ± 230* | 25.3 ± 4.5 (366 ± 122 mg) | 15.7 ± 3.7 (201 ± 30 mg) |
| Renal artery    | 12 1596 ± 323* | 53.3 ± 9.1 (826 ± 63 mg) | 11.1 ± 1.9* (176 ± 36 mg) |
| Femoral artery  | 7 3770 ± 502* | 113 ± 7.7 (4185 ± 875 mg) | 61.1 ± 12.3# (1937 ± 332 mg) |
| Mesenteric vein | 9 857 ± 189** | 94.4 ± 13.0 (821 ± 138 mg) | 51.2 ± 9.8# (464 ± 77 mg) |

*Contraction caused by 30 mM K⁺; **contraction caused by 5 mM Ba⁺⁺. N, number of monkeys used. Values in parentheses indicate mean absolute contractions. # Relative to the contraction induced by 30 mM K⁺ or 5 mM Ba⁺⁺. # #Stabilized contraction after repeated ANG II trials. Significantly different from the value in the first trial: *P < 0.001, #P < 0.01, #P < 0.02.
Fig. 1. Contractile responses to $10^{-7}$ M ANG II of coronary artery, mesenteric vein and renal artery strips from a monkey before, during and after treatment with $10^{-6}$ M indomethacin.

Fig. 2. Modification by $10^{-6}$ M indomethacin (IM), $5 \times 10^{-5}$ M aspirin (Asp.) and endothelium denudation [E(-)] of the response to $10^{-7}$ M ANG II in monkey coronary arteries. The responses before treatment with the inhibitors or in the arteries with endothelium (C) were taken as 100%; mean absolute values were $209 \pm 48$ mg (n = 14), $223 \pm 83$ mg (n = 4) and $173 \pm 27$ mg (n = 7), respectively. W = after repeated washing of the strips with drug-free fluid. Significantly different from the control: $^aP < 0.01$, $^hP < 0.02$ (paired comparison). Numbers in the parentheses indicate the number of monkeys used. Vertical bars represent S.E.M.

Fig. 3. Modification by $10^{-6}$ M indomethacin (IM) and $5 \times 10^{-5}$ M aspirin (Asp.) of the response to $10^{-7}$ M ANG II in monkey renal and femoral arteries and mesenteric veins. The responses before treatment with the inhibitors (C) were taken as 100%; mean absolute values were $176 \pm 36$ mg (n = 12), $1937 \pm 332$ mg (n = 7) and $464 \pm 77$ mg (n = 9), respectively. Significantly different from the control: $^aP < 0.02$, $^bP < 0.05$. Numbers in the parentheses indicate the number of monkeys used.
tion before and after indomethacin was added were 1.26 ± 0.11 and 2.09 ± 0.27 min (n = 9, P < 0.02), respectively.

Femoral artery responses to ANG II were not influenced by treatment with indomethacin (Fig. 3). The duration of contraction was not significantly increased (from 2.85 ± 0.27 to 4.90 ± 0.97 min, n = 6).

DISCUSSION

Tachyphylaxis rapidly developed by repeated applications of ANG II in monkey arteries and veins, as seen in blood vessels from other mammals. However, modifications by indomethacin and aspirin of the peptide-induced contraction differed in these monkey blood vessels: a potentiation in the renal arteries, no change in the femoral arteries and mesenteric veins, and an attenuation in the coronary arteries. Therefore, cyclooxygenase products do not appear to participate in the development of tachyphylaxis in coronary and femoral arteries and mesenteric veins.

In coronary arteries, contractions induced by ANG II were moderately inhibited by treatment with indomethacin and aspirin, as observed in monkey cerebral arteries, in which the inhibition is more evident (14). Similar inhibition was also obtained in isolated, perfused cat coronary arteries (17). The peptide-induced contractions were less in the endothelium-denuded arteries than in the intact arteries; the inhibitory effect of indomethacin was similar in the arteries with and without endothelium. Treatment with OKY 046 in a concentration (10^-5 M) sufficient to markedly suppress the activity of thromboxane A2 (TXA2) synthetase in rabbit platelets (18) did not significantly alter the response to ANG II. Therefore, the coronary artery contraction caused by the peptide is postulated to derive from the release of prostaglandins, such as PGF2α, PGE2 and PGD2, but not TXA2, from the endothelium or from subendothelial tissues. According to Gunther and Cannon (19), increased levels by intravenous ANG II infusion of PGF2α and PGE2 in coronary artery sinus plasma in anesthetized dogs are diminished by treatment with indomethacin. PGF2α and carbocyclic TXA2, an analog of TXA2 (20), PGE2 and PGD2 (N. Toda, unpublished data) are potent constrictors of monkey coronary arteries. Indomethacin and aspirin in high concentrations (16) did not abolish the peptide-induced contraction (40 to 50% contraction left after the treatment, Fig. 2), and saralasin abolished the response of the arteries in the presence and absence of cyclooxygenase inhibitors. It appears that ANG II also acts directly on its receptors in coronary artery smooth muscle to initiate contraction.

Monkey renal arteries responded to ANG II with a transient contraction that was potentiated and prolonged by treatment with indomethacin and aspirin. Similar but more marked alterations were seen in monkey mesenteric arteries treated with cyclooxygenase inhibitors (14). PGF2α appears to be released, blunting the vasoconstriction caused by stimulation of ANG II receptors in smooth muscle. The duration of contraction in mesenteric veins was significantly increased by indomethacin. Vasodilator PGs seem to be involved also in the venous response. Femoral artery contractions were not altered by the cyclooxygenase inhibitor. The vasoconstrictor action of PGs released by the peptide may be balanced by the action of vasodilator PGs, such as PGF2α, or ANG II may not release PGs from these blood vessels in amounts eliciting significant responses. Many studies reported so far indicate the release of PGs from a variety of blood vessels from different mammals in response to ANG II (6, 21–24); therefore, the latter alternative is less likely. Figure 4 summarizes the effect of indomethacin on the ANG II-induced contraction in various blood vessels from monkeys, obtained in the present and previous (14) studies. In the coronary and cerebral arteries, the contraction was suppressed or abolished by the inhibitor, whereas the response was increased in mesenteric and renal arteries. These divergent results appear to be associated with the different ability of the blood vessels to produce vasoconstrictor/
vasodilator PGs in endothelial and subendothelial tissues and with the different responsiveness of smooth muscle to the PGs released. In fact, PGI2-induced relaxations are less in monkey cerebral and coronary arteries than in these arteries from dogs and humans (20, 25) and also monkey mesenteric and renal arteries (N. Toda, unpublished data).

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