MODIFICATION OF ANGIOTENSIN II-INDUCED RELAXATION
BY DIPYRIDAMOLE, PHTHALAZINOL AND ASPIRIN IN
ISOLATED DOG RENAL ARTERIES

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Abstract—Angiotensin (ANG) II-induced relaxations in isolated dog renal and cerebral arteries are postulated to be mediated by the release of prostaglandin (PG) I₂ from the arterial wall. In helical strips of dog renal arteries treated with dipyridamole, relaxations induced by ANG II (10⁻⁷ M) and exogenously applied PGI₂ (10⁻⁸ M) were potentiated; the potentiation was appreciably greater in the ANG-induced relaxation. Treatment with phthalazinol did not alter the response to ANG II, but significantly potentiated the relaxation induced by PGI₂. The ANG-induced relaxations were suppressed or reversed to contractions by aspirin or indomethacin. Combined treatment of dipyridamole with aspirin or indomethacin restored the relaxant response to ANG II, while phthalazinol in combination with aspirin did not restore the response. It may be concluded that the potentiation of responses to ANG II by dipyridamole is associated with increments in the release of PGI₂ from the arterial wall and potentiations of the response of arterial smooth muscle to PGI₂. Dipyridamole appears to increase the production of PGI₂ even in the presence of PG synthesis inhibition by aspirin or indomethacin.

Aspirin inhibits the aggregation of platelets induced by collagen, adrenaline and ADP due to inactivation of cyclooxygenase which transforms platelet arachidonic acid to prostaglandin (PG) endoperoxides (1, 2). Results of the efficacy of aspirin in prevention of ischemic cerebral and myocardial diseases are not yet conclusive (3, 4). The inhibition by aspirin of PGI₂ biosynthesis in the vascular wall may reduce its effectiveness on the diseases (5). Combined treatment with dipyridamole and aspirin reduced coronary mortality (6). These antithrombotic agents have different and additive modes of action (7). However, attention has not been paid to the action of dipyridamole on an aspirin-induced inhibition of the PGI₂ synthesis.

Angiotensin (ANG) II produces a relaxation of isolated dog renal and cerebral arteries contracted with PGF₂α, PGE₂ or norepinephrine (8–10). This relaxation is abolished by ANG II antagonists, reversed to a contraction by aspirin or indomethacin, and suppressed by PGI₂ synthesis inhibitors, 15-hydroperoxy arachidonic acid and tranylcypromine. In the superfusate of isolated renal arteries in response to ANG II, PGI₂-like substance is detected by the bioassay system with rat stomach and dog coronary artery strips (9). It is thus postulated that ANG II stimulates ANG receptors in the vascular wall, resulting in an increment of the synthesis and the release of PGI₂ which relaxes the arterial strip (9, 10). The ANG-induced release of PGI₂-like substance from blood vessels has also been demonstrated (11, 12).

The present study was thus undertaken to determine the alteration by dipyridamole and
phthalazinol of the relaxant response of isolated dog renal arteries to ANG II, possibly mediated via endogenous PGI$_2$, and the response to exogenously applied PGI$_2$. Dipyridamole and phthalazinol inhibit the aggregation of platelets, possibly by an accumulation of cyclic AMP in the platelets (13, 14). Interaction between dipyridamole and aspirin or indomethacin in the ANG-induced relaxations was also studied.

Materials and Methods

Mongrel dogs of either sex, weighing 7 to 15 kg, were anesthetized with intraperitoneal injections of sodium thiopental (50 mg/kg) and sacrificed by bleeding from the common carotid arteries. The kidney was rapidly removed. Intrarenal, interlobar branches of the renal artery (0.5 to 0.8 mm outside diameter) were isolated. The arteries were helically cut into strips, approx. 20 mm long. The strips were vertically fixed between hooks in a muscle bath containing the nutrient solution, which was aerated with a mixture of 95% O$_2$ and 5% CO$_2$ and maintained at 37±0.3°C. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer (Nihon Kohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 1.5 g. Constituents of the solutions were as follows (mM): Na$^+$, 144.8; K$^+$, 5.4; Ca$^{2+}$, 2.2; Mg$^{2+}$, 1.0; Cl$^-$, 131.6; HCO$_3^-$, 25.0; and dextrose, 5.6. The pH of the solution was 7.34 to 7.41. Before the start of experiments, the arterial strips were allowed to equilibrate for 60 to 90 min in the bathing media. During the equilibration period, the bathing solutions were replaced every 10 to 15 min. Isometric contractions and relaxations were recorded on an ink-writing oscillograph (Sanei Sokki Co., Tokyo, Japan). The contractile response to 30 mM K$^+$ was first obtained. Preparations were washed three times with control media and equilibrated for 40 to 50 min, during which time the bathing media were replaced every 10 to 15 min. Arterial strips were contracted with prostaglandin (PG) F$_{2\alpha}$ (10$^{-7}$ to 5×10$^{-7}$ M): the contraction was in a range between 25 and 40% of the contraction induced by 30 mM K$. ANG II in a concentration of 10$^{-7}$ M was added; and after the tension was returned and stabilized, PGI$_2$ (10$^{-6}$ M) was added. At the end of each series of experiments, papaverine in a concentration of 10$^{-4}$ M was added to attain the maximum relaxation (15); relaxations induced by ANG II and PGI$_2$ relative to those induced by papaverine are presented. When contractions were induced by ANG II, contractions induced by 30 mM K$^+$ minus PGF$_{2\alpha}$-induced contractions were taken as 100%. The responses to ANG II and PGI$_2$ were obtained three times to confirm the reproducibility. The third responses were taken as controls. After the third trials, tachyphylaxis did not develop (9). Arterial strips had been treated for 60 min with polyphloretin phosphate or for 30 min with the other blocking or test drugs before ANG II was added. Concentration-response curves for PGI$_2$ were obtained by adding the PG to the bathing media in cumulative concentrations. The results shown in the text, figures and table were expressed as mean values±S.E.M. Statistical analyses were made using Student's t-test.

Drugs used were angiotensin II (ANG II, Protein Research Foundation, Osaka), prostaglandin I$_2$ sodium (Ono Pharmaceutical Co., Osaka), dipyridamole (Persantin, C. H. Boehringer, Ingelheim), phthalazinol (EG626, 7 ethoxycarbonyl-6,8-dimethyl-4-hydroxy-methyl-1(2H)-phthalazinone, Banyu Pharmaceutical Co., Tokyo); acetyl salicylic acid (aspirin), indomethacin, polyphloretin phosphate (Ono Pharmaceutical Co.), prostaglandin F$_{2\alpha}$, and papaverine hydrochloride. The stock solution of dipyridamole in an ampoule (10 mg/2 ml) was diluted with
distilled water before use. The solvent of dipyridamole (C. H. Boehringersohn) was also diluted as was the dipyridamole solution.

Results

Effects of dipyridamole and phthalazinol on the response to ANG II and PGI₂: In helical strips of dog renal arteries contracted with PGF₂α, the addition of ANG II in a concentration of 10⁻⁷ M caused a transient contraction followed by a moderate relaxation. Exogenously applied PGI₂ (10⁻⁸ M) moderately relaxed the renal arterial strips. Treatment with 10⁻⁵ M dipyridamole potentiated the relaxant response to ANG II by 100% (Fig. 1) and reduced the contractile response from 199±39 mg to 89±18 mg (N=33, P<0.02). In 9 strips in which ANG II produced only a slight (less than 30 mg) or no contraction but a moderate relaxation, dipyridamole potentiated the relaxant response (from 33.8±6.5% to 64.7±6.4%) to a similar extent to the response of arteries showing greater contractions induced by ANG II (from 31.1±4.7% to 63.1±3.7%, N=24). The PGI₂-induced relaxation was also potentiated by dipyridamole; however, the potentiation was less than that seen in the response to ANG II (22% vs. 100%, Fig. 1).

In paired comparison, dipyridamole always potentiated the ANG-induced relaxation to a greater extent. The potentiating effect was reversed by repeated washing of preparations. Typical responses to ANG II and PGI₂ before and after treatment with dipyridamole are shown in the upper tracings of Fig. 2. The solvent of dipyridamole in the same concentration as that obtained when 10⁻⁵ M dipyridamole was applied did not alter the relaxant response to ANG II (Table 1) and PGI₂ (43.5±8.4% in the control and 42.0±8.0% in strips treated with solvent, N=6). On the other hand, phthalazinol (10⁻⁵ M) did not alter the relaxation induced by ANG II, but significantly potentiated the PGI₂-induced relaxation (Fig. 2, lower tracings, and Fig. 3). The contractile response to ANG II tended to be attenuated (from 149±47 mg to 104±35 mg, N=16). The concentration-response curve for PGI₂ was shifted to the left following treatment with 10⁻⁵ M phthalazinol. Treatment for 60 min with polyphloretin phosphate (3×10⁻⁶ g/ml), an antagonist of vasoconstrictor PG's (16), did not significantly affect the relaxation induced by ANG II (Table 1) and PGI₂.

In renal arterial strips contracted with PGF₂α, dipyridamole (10⁻⁵ M) produced a transient relaxation; tachyphylaxis of the response rapidly developed. Paired comparisons of the relaxant response to dipyridamole were made in control arteries...
Fig. 2. Responses of renal arterial strips to ANG II and PGI₂ in the absence and presence of treatment with dipyridamole (upper tracings) or phthalazinol (lower tracings). Horizontal lines just left of each tracing represent the level prior to the addition of PGF₂α. PA=10⁻⁴ M papaverine.

Table 1. Modification by drugs of the response to ANG II (10⁻⁷ M) of renal arterial strips contracted with PGF₂α.

| Treatment                      | N  | Responses (%) * to ANG II | Significance |
|--------------------------------|----|---------------------------|--------------|
|                                |    | Control                  | Experimental |               |
| Dipyridamole, 10⁻⁶ M           | 33 | -31.8±3.8                | -63.6±3.1    | P<0.001       |
| Dipyridamole solvent           | 8  | -53.0±4.2                | -51.9±5.5    | N.S.          |
| Polypheolizin, 3x10⁻⁵ g/ml      | 8  | -62.1±4.4                | -42.5±7.8    | N.S.          |
| Aspirin, 2x10⁻⁶ M              | 8  | -49.4±4.3                | -42.3±6.8    | N.S.          |
| Aspirin, 10⁻⁵ M                | 14 | -46.1±5.4                | -12.5±9.9    | P<0.001       |
| Aspirin, 5x10⁻⁵ M              | 11 | -51.2±6.4                | +30.3±6.0    | P<0.001       |
| Indomethacin, 3x10⁻⁸ M         | 10 | -61.8±6.1                | -7.5±6.5     | P<0.001       |
| Indomethacin, 10⁻⁷ M           | 4  | -66.5±8.5                | +9.8±5.6     | P<0.001       |
| Aspirin, 10⁻⁶ M                | 11 | -46.4±7.1                | -24.2±9.2    | N.S.          |
| +Dipyridamole, 10⁻⁵ M          |    |                          |              |               |
| Aspirin, 5x10⁻⁵ M              | 5  | -41.8±4.3                | +3.8±2.1     | P<0.001       |
| +Dipyridamole, 10⁻⁵ M          |    |                          |              |               |
| Aspirin, 10⁻⁵ M                | 5  | -51.0±7.6                | +8.6±4.2     | P<0.001       |
| +Phthalazinol, 10⁻⁶ M          |    |                          |              |               |
| Indomethacin, 3x10⁻⁶ M         | 10 | -65.2±5.2                | -33.6±6.5    | P<0.01        |
| +Dipyridamole, 10⁻⁵ M          |    |                          |              |               |

- Relaxation; +, Contraction. *Values relative to relaxations induced by 10⁻⁴ M papaverine or to contractions induced by 30 mM K⁺ minus PGF₂α-induced contractions. N, number of preparations used; N.S., not significant (P>0.05).

and those treated with 5x10⁻⁵ M aspirin; mean values of the relaxation relative to the papaverine (10⁻⁴ M)-induced relaxation were 59.0±6.3% and 58.0±5.9% (N=5), respectively.

Interaction between dipyridamole and cyclooxygenase inhibitors: Relaxations induced by 10⁻⁷ M ANG II were suppressed or
Fig. 3. Modification by phthalazinol (EG626) of relaxant responses of renal arterial strips to ANG II (left figure) and PGI2 (right figure). The strips were contracted with PGF2α. Relaxations induced by 10⁻⁴ M papaverine were taken as 100%; mean absolute values in control and phthalazinol-treated strips in response to ANG II were 715±62 mg and 560±67 mg (N=16), respectively, and the values in the strips in response to PGI2 were 811±89 mg and 681±92 mg (N=16), respectively. a, Significantly different from the control, P<0.001. C=control, Phthal. =10⁻⁵ M phthalazinol.

reversed to contractions following treatment of renal arterial strips with aspirin in concentrations higher than 10⁻⁵ M or with indomethacin in concentrations higher than 3×10⁻⁸ M (Table 1). Dipyridamole (10⁻⁵ M) applied in combination with 10⁻⁵ M aspirin markedly attenuated the inhibitory effect of aspirin (Fig. 4); responses to ANG II in arteries treated with aspirin alone and with aspirin plus dipyridamole differed significantly (12.5% contraction vs. 24.2% relaxation, respectively, P<0.02) (Table 1). Combined treatment with dipyridamole and aspirin potentiated the relaxation induced by PGI2. However, the inhibitory effect of 5×10⁻⁵ M aspirin was not prevented by dipyridamole in concentrations of 10⁻⁵ (Table 1) and 5×10⁻⁵ M. Relaxations induced by ANG II in arterial strips treated with dipyridamole (10⁻⁵ M) and indomethacin (3×10⁻⁸ M) were appreciably greater than those seen in arteries treated with indome-
Fig. 5. Modification of relaxant responses of renal arterial strips to ANG II and PGI$_2$ by indomethacin alone (upper figures) or indomethacin plus dipyridamole (lower figures). The strips were contracted with PGF$_2$-$\alpha$. Relaxations induced by $10^{-4}$ M papaverine were taken as 100%; mean absolute values in control and indomethacin-treated strips in response to ANG II (upper left) and the strips in response to PGI$_2$ (upper right) were 755±56 mg, 896±62 mg (N=10), 835±76 mg and 1005±70 mg (N=10), respectively, and the values in control and {indomethacin+dipyridamole}-treated strips in response to ANG II (lower left) and the strips in response to PGI$_2$ (lower right) were 770±40 mg, 829±83 mg (N=10), 822±45 mg and 897±79 mg (N=8), respectively. a, Significantly different from the control, P<0.001; b, P<0.01. C=control, Indo. =3X10$^{-8}$ M indomethacin, Indo. +Dypyr. =combined treatment with 3X10$^{-8}$ M indomethacin and 10$^{-5}$ M dipyridamole.

Fig. 6. Responses of a renal arterial strip to $10^{-7}$ M ANG II and $10^{-8}$ M PGI$_2$ before and after treatment with indomethacin alone or indomethacin plus dipyridamole. Horizontal lines just left of each tracing represent the level prior to the addition of 3X10$^{-7}$ M PGF$_2$-$\alpha$. PA=10$^{-4}$ M papaverine.

Treatment of renal arterial strips with dipyridamole potentiated the relaxant response to ANG II, which is postulated to result mainly from PGI$_2$ released from the arterial wall (9, 10). In preparations in response to ANG II in which only a slight or no contraction preceded the relaxation, dipyridamole potentiated the relaxation to a
similar extent to that seen when the contraction was evident. When the contractile response to ANG II was inhibited by phthalazinol, the relaxation was not potentiated (Fig. 2, lower tracings). Polyphloretin phosphate did not potentiate the relaxation. ANG II has been demonstrated to release PGI₂ as well as vasoconstrictor PG's such as PGF₂α and E₂ (9); the contractile response to these PG's is specifically attenuated by polyphloretin in the concentration used here (16). This antagonist, however, does not reduce the relaxant response to PGI₂ (Toda, unpublished data). These results indicate that the potentiation of relaxant responses to ANG II by dipyridamole is not due to an inhibition of the contraction. The arterial relaxation induced by PGI₂ was also potentiated by dipyridamole as was the antiaggregating action of PGI₂ (7); however, the potentiation in the arteries was appreciably less than that seen in the response to ANG II. Therefore, it appears that the increased biosynthesis and release of PGI₂ as well as the augmented responsiveness of arterial smooth muscle to PGI₂ are involved in the potentiation of ANG-induced relaxations. The biosynthesis of PGI₂ from tritiated arachidonic acid in rat stomach fundus homogenates and from PGH₂ in pig aorta microsomes is reportedly increased by dipyridamole (17).

Dipyridamole produced an arterial relaxation, which was unaffected by treatment with aspirin, indicating that vasodilator PG's are not involved in the dipyridamole-induced relaxation. It may be concluded that dipyridamole does not release precursor fatty acid, but increases the biosynthesis of PGI₂ when the precursor is released by chemical stimuli such as ANG II or when arachidonic acid or PGH₂ is applied (17).

Treatment with phthalazinol potentiated the relaxation induced by PGI₂, but not the relaxant response to ANG II. Similar potentiation of the effect of exogenously applied PGI₂ has been seen in rabbit platelets (18). Therefore, phthalazinol may interfere either with the biosynthesis or the release of PGI₂ stimulated by ANG II in dog renal arteries or may inhibit the action of ANG II on receptors. Dipyridamole and phthalazinol inhibit cyclic AMP phosphodiesterase (13, 19) and accumulate cyclic AMP in platelets, being possibly involved in the inhibition of platelet aggregation (13). PGI₂ increases the production of cyclic AMP in arteries (20) and has antiaggregating (21) and vasodilator activities (22). Therefore, potentiating effects of dipyridamole and phthalazinol may be explained by a greater accumulation of cyclic AMP in dog renal arterial smooth muscle. However, effects of these antiaggregating agents on the arterial relaxation induced by ANG II were quite different. Therefore, it seems unlikely that changes in the synthesis of PGI₂ and its release from the arterial wall are related to the phosphodiesterase inhibition and the cyclic AMP accumulation.

ANG II-induced relaxations were abolished or reversed to contractions following treatment with aspirin or indomethacin, which is expected to suppress the production of PGI₂ following the inhibition of cyclooxygenase activity. Dipyridamole prevented the inhibitory effect of aspirin and indomethacin in concentrations sufficient to partially inactivate the enzyme. The prevention could be associated mainly with the increased biosynthesis and release of PGI₂ and to a lesser extent with the augmented responsiveness of arteries to PGI₂.

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References

1) Ferreira, S.H. and Vane, J.R.: New aspect of the mode of action of non-steroid antiinflammatory drugs. Annu. Rev. Pharmacol. 14, 57–73 (1974)

2) Flower, R.J.: Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev. 26, 33–67 (1974)

3) Fields, W.S., Lemak, N.A., Frankowski, R.F. and Hardy, R.J.: Controlled trial of aspirin in cerebral ischemia. Stroke 8, 301–314 (1977)

4) Hennekens, C.H., Karlson, L.K. and Rosner, B.: A case-controlled study of regular aspirin use and coronary deaths. Circulation 58, 35–38 (1978)

5) Moncada, S. and Vane, J.R.: Arachidonic acid metabolites and the interactions between platelets and blood vessel walls. N. Engl. J. Med. 300, 1142–1147 (1979)

6) Persantin-Aspirin Reinfarction Study Research Group (PARIS): Persantin and aspirin in coronary heart disease. Circulation 62, 449–461 (1980)

7) Moncada, S. and Korbut, R.: Dipyridamole and other phosphodiesterase inhibitors act as antithrombotic agents by potentiating endogenous prostacyclin. Lancet 1, 1286–1289 (1978)

8) Toda, N.: Heterogeneity in the relaxation of vascular smooth muscle. In Mechanisms of Vasodilatation, Edited by Vanhoutte, P. M. and Leusen, I., p. 129–136, S. Karger, Basel (1980)

9) Toda, N. and Miyazaki, M.: Angiotensin-induced relaxation in isolated dog renal and cerebral arteries. Am. J. Physiol. 240, H247–H254 (1981)

10) Toda, N.: Mechanism of vascular smooth muscle relaxation induced by angiotensins. In Vasodilation, Edited by Vanhoutte, P. M. and Leusen, I., p. 153–159, Raven Press, New York (1981)

11) Puré, E. and Needleman, P.: Effect of endothelial damage on prostaglandin synthesis by isolated perfused rabbit mesenteric vasculature. J. Cardiovasc. Pharmacol. 1, 299–309 (1979)

12) Dusting, G.J., Mullins, E.M. and Nolan, R.D.: Prostacyclin (PGI₂) release accompanying angiotensin conversion in rat mesenteric vasculature. Eur. J. Pharmacol. 70, 129–137 (1981)

13) Mills, D.C.B. and Smith, J.B.: The influence on platelet aggregation of drugs that affect the accumulation of adenosine 3',5'-cyclic monophosphate in platelets. Biochem. J. 121, 185–196 (1971)

14) Tanaka, K., Harada, Y., Iwata, M. and Katori, M.: Potentiation of antiaggregating activity of PGI₂ by 7 ethoxycarbonyl-6,8-dimethyl-4-hydroxymethyl-1(2H)-phthalazinone (EG626) in rabbit platelets in vitro. Prostaglandins 20, 255–268 (1980)

15) Toda, N.: The action of vasodilating drugs on isolated basilar, coronary and mesenteric arteries of the dog. J. Pharmacol. Exp. Ther. 191, 139–146 (1974)

16) Toda, N. and Miyazaki, M.: Responses of isolated dog cerebral and peripheral arteries to prostaglandins after application of aspirin and polyphloretin phosphate. Stroke 9, 490–498 (1978)

17) Blass, K.-E., Block, H.-U., Forster, W. and Pönicke, K.: Dipyridamole: A potent stimulator of prostacyclin (PGI₂) biosynthesis. Br. J. Pharmacol. 68, 71–73 (1980)

18) Tanaka, K., Harada, Y. and Katori, M.: EG-626: Not a thromboxane A₂ antagonist, but a PGI₂ potentiator in platelet aggregation. Prostaglandins 17, 235–237 (1979)

19) Adachi, K. and Numano, R.: Phosphodiesterase inhibition: Their comparative effectiveness in vitro in various organs. Japan. J. Pharmacol. 27, 97–103 (1977)

20) Kukovetz, W.R., Holzmann, S., Wurm, A. and Pöch, G.: Prostacyclin increases cAMP in coronary arteries. J. Cyclic Nucleotide Res. 5, 469–476 (1979)

21) Moncada, S., Gryglewski, R., Bunting, S. and Vane, J.R.: An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 263, 663–665 (1976)

22) Dusting, G.J., Moncada, S. and Vane, J.R.: Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid. Prostaglandins 13, 3–15 (1977)