Effect of Forskolin on Prostaglandin Productions in Isolated Dog Renal Arteries

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Abstract—Forskolin (1 to 100 nM), a direct activator of adenylate cyclase, did not have any effect on prostaglandin E2 and I2 productions in isolated dog renal arteries. However, forskolin at the lower concentrations (10 and 100 nM) markedly stimulated only prostaglandin E2 production. 8-Bromo-cyclic AMP (0.5 and 1 mM) failed to stimulate prostaglandin E2 and I2 productions. The results suggest that 1) forskolin stimulates only prostaglandin E2 production, not through the activation of adenylate cyclase and 2) the prostaglandin production system may be independent of the cyclic AMP-generating system in isolated dog renal arteries.

Forskolin is a powerful activator of adenylate cyclase and a potent vasodilator (1, 2). This vasodilation of forskolin seems to derive from its direct activation of adenylate cyclase (3). On the other hand, cyclic AMP has been reported to regulate the activity of phospholipase and/or cyclooxygenase (4–6). In this experiment, we have investigated the effect of forskolin on PGE2 and I2 productions in isolated dog renal arteries. In order to further elucidate the relationship between the stimulations of adenylate cyclase and PG productions in the dog renal arteries, we have used 8-bromo-cyclic AMP. Interestingly enough, forskolin markedly stimulated PGE2 production in the lower concentrations, while in the higher concentrations, forskolin failed to enhance it. 8-Bromo-cyclic AMP also did not have any effect on PGE2 and I2 productions. The results obtained here suggest that the PG production system seems not to be regulated by cyclic AMP in the dog renal arteries, while forskolin stimulates only PGE2 production, not through the activation of adenylate cyclase.

The renal arteries were isolated from mongrel dogs of either sex weighing 7 to 11 kg under anesthesia with sodium pentobarbital (30 mg/kg, i.v.). The obtained dog renal arteries were carefully removed free from surrounding tissues in ice-cold oxygenated Krebs-Henseleit buffer solution. Then the cleaned renal arteries were cut into ring segments (approximately 3 mm in length). The strips were incubated with Krebs-Henseleit buffer solution at 37°C under 95% O2-5% CO2. After the pre-incubation for 60 min, the strips were pre-treated for 30 min with forskolin (Calbiochem.) or 8-bromo-cyclic AMP (Sigma). At the end of the further 30 min incubation with or without each drug, the incubation medium was sampled. The concentrations of PGE2 and PG12 (determined as 6-keto-PGF1a) were measured by a method of radioimmunoassay, which was previously described (7).

Forskolin, a direct activator of adenylate cyclase, has been shown to elevate intracellular cyclic AMP levels in a dose-dependent manner (1 to 100 nM) (1, 3). In these concentrations, forskolin did not have any effect on the spontaneous PGE2 and 6-keto-PGF1a productions in isolated dog renal arteries (Fig. 1). However,
forskolin at the lower concentrations (10 and 100 nM) markedly stimulated the spontaneous PGE2 production, while it failed to stimulate the spontaneous 6-keto-PGF1α production (Fig. 1). We have previously shown that the vascular endothelial cells are the major sources of PG12 in the dog renal arteries, while PGE2 is mainly produced in other cell types, perhaps vascular smooth muscle cells (8). Therefore, this difference of the stimulatory effect of forskolin may be related to the difference of the synthetic sites of PG.

To test the relationship between cyclic AMP and PG productions, we have further used 8-bromo-cyclic AMP, one of the cyclic AMP derivatives. 8-Bromo-cyclic AMP (0.5 and 1 mM) did not have any effect on PGE2 and 6-keto-PGF1α productions (Table 1). Furthermore, we noticed PGE2 and 6-keto-PGF1α productions were not different from the control (4.37±0.13 and 53.46±4.28 ng/g wet weight, respectively) after the treatment with 10 μM isoproterenol (4.59±1.40 and 59.46±4.28 ng/g wet weight, respectively; n=3), which activates adenylate cyclase through the beta-receptor. These results suggest that the PG production system will be independent of the cyclic AMP-generating system in the dog renal arteries. This suggestion is supported by Brotherton et al. (9) and Whorton et al. (10) who have been reported that forskolin failed to stimulate PG12 production in spite of increasing the intracellular levels of cyclic AMP in the human umbilical vein and porcine

Table 1. Effect of 8-bromo-cyclic AMP on PGE2 and 6-keto-PGF1α productions in isolated dog renal arteries

|                      | PG productions (ng/g wet weight) |
|----------------------|----------------------------------|
|                      | PGE2                             | 6-keto-PGF1α                      |
| Control              | 7.72±1.64                        | 34.87±2.92                        |
| 8-bromo-cyclic AMP    | 0.5 mM                           | 35.73±5.15                        |
|                      | 1 mM                             | 39.95±5.93                        |

Values represent the mean±S.E. (n=6). The strips were treated with 8-bromo-cyclic AMP for 30 min after the pre-incubation for 60 min. Then the strips were incubated for a further 30 min with 8-bromo-cyclic AMP.
aortic endothelial cells (9, 10). Therefore, our results obtained here further suggest that the stimulatory effect of forskolin at the lower concentrations on PGE2 production seems to be the result of some other, cyclic AMP-independent action of the drug, possibly related to the activation of PGE2 isomerase.

References
1 Seamon, K.B., Padgett, W. and Daly, J.W.: Forskolin: Unique diterpene activator of adenylate cyclase in membranes and in intact cells. Proc. Natl. Acad. Sci. U.S.A. 78, 3363–3367 (1981)
2 Linder, E., Dohadwalla, A.N. and Bhattacharya, B.K.: Positive inotropic and blood pressure lowering activity of a diterpene derivative isolated from coleus forskohli: Forskolin. Arzneimittel-forsch. 28, 284–289 (1978)
3 Seamon, K.B. and Daly, J.W.: Forskolin: a unique diterpene activator of cyclic AMP-generating systems. J. Cyclic Nucleotide Res. 7, 201–224 (1981)
4 Minker, M., Stanford, N., Chi, M.-Y., Roth, G.J., Raz, A., Needleman, P. and Majerus, P.W.: Cyclic adenosine 3',5'-monophosphate inhibits the availability of arachidonate to prostaglandin synthesis in human platelet suspensions. J. Clin. Invest. 59, 449–454 (1977)
5 Lapetina, E.G., Schmitger, C.J., Chandrabase, K. and Cuatrecasas, P.: Cyclic adenosine 3',5'-monophosphate and prostacyclin inhibit membrane phospholipase activity in platelets. Biochem. Biophys. Res. Commun. 76, 828–835 (1977)
6 Gorman, R.R., Wierenga, W. and Miller, O.V.: Independence of the cyclic AMP-lowering activity of thromboxane A2 from the platelet release reaction. Biochim. Biophys. Acta 572, 95–104 (1979)
7 Satoh, H., Hosono, M. and Satoh, S.: Distinctive effect of angiotensin II on prostaglandin production in dog renal and femoral arteries. Prostaglandins 27, 807–820 (1984)
8 Satoh, H. and Satoh, S.: Prostaglandin E2 and I2 production in isolated dog renal arteries in the absence or presence of vascular endothelial cells. Biochem. Biophys. Res. Commun. 118, 873–876 (1984)
9 Brotherton, A.F.A., Macfarlane, D.E. and Hoak, J.C.: Prostaglandin biosynthesis in vascular endothelium is not inhibited by cyclic AMP. Studies with 3-isobutyl-1-methylxanthine and forskolin. Thromb. Res. 28, 637–647 (1982)
10 Whorton, A.R., Collawn, J.B., Montgomery, M.E., Young, S.L. and Kent, R.S.: Arachidonic acid metabolism in cultured aortic endothelial cells. Effect of cAMP and 3-isobutyl-1-methylxanthine. Biochem. Pharmacol. 34, 119–123 (1985)