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Effect of angiotensin-converting enzyme inhibitor YS980 on prostaglandin synthesis in rabbit kidney medulla slices

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The effect of YS980, an angiotensin-converting enzyme inhibitor, on the generation of medullary prostaglandins E_2 and $F_{2\alpha}$ was examined. At concentrations of 0.2 mM and below, YS980 enhanced prostaglandin $F_{2\alpha}$ synthesis at the expense of prostaglandin E_2. At concentrations of 0.4 mM or more, YS980 inhibited synthesis of both prostaglandins E_2 and $F_{2\alpha}$ markedly. These results suggest that YS980 has the potential to modulate prostaglandins E_2 and $F_{2\alpha}$ synthesis by the kidney and that this effect may represent some pharmacological action of the drug.

Introduction It has been reported that (4**R**)-3-[(2S)-3-mercapto-2-methylpropanoyl]-4-thiazolidinecarboxylic acid (YS980), a sulphhydryl compound, is a novel potent angiotensin-converting enzyme inhibitor (Mita *et al.*, 1978; Iso *et al.*, 1978). Angiotensin-converting enzyme converts the biologically inactive angiotensin I to the potent vasopressor form angiotensin II and also degrades bradykinin, a depressor peptide (Dorer *et al.*, 1974; Erdos, 1976). Thus, inhibitors of angiotensin-converting enzyme have been shown to be useful for renal hypertension (Ondetti *et al.*, 1977; Cushman *et al.*, 1977). Recently, we have reported that sulphhydryl compounds such as reduced glutathione and cysteine play an important role in the control of prostaglandins E_2 and $F_{2\alpha}$ synthesis in renal medulla (Fujita *et al.*, 1986). The renal medulla is very rich in prostaglandins as well as in the enzymes that biosynthesize prostaglandins. Intrarenal prostaglandins seem to be important determinants of blood pressure (Tobian & O'Donnell, 1976). These findings prompted us to examine the effect of YS980 on the *in vitro* generation of medullary prostaglandins E_2 (PGE_2) and $PGF_{2\alpha}$.

Methods Kidney medulla slices were prepared from male rabbits (2–2.5 kg) as described elsewhere (Fujimoto & Fujita, 1982). In all experiments, the slices (0.4 g) were preincubated in 4.0 ml of 0.15 M KCl/0.02 M Tris-HCl buffer (pH 7.4) at 4°C for 5 min. Following preincubation, the medium was discarded, the slices rinsed twice with the Tris-HCl buffer and incubated with the indicated concentrations of YS980 (Santen Pharmaceuticals Ltd., Japan) or aspirin at 37°C for 30 min.

We showed previously that the major prostaglandins produced in our incubation of medulla slices and recovered in the medium were PGE_2 and $PGF_{2\alpha}$ (Fujimoto *et al.*, 1983). PGE_2 and $PGF_{2\alpha}$ in the incubation medium were simultaneously determined by a high performance liquid chromatographic (h.p.l.c.) method as described by Fujita *et al.* (1986). Briefly, PGE_2 and $PGF_{2\alpha}$ extracted with ethyl ether (approximately pH 3) were measured after esterification of prostaglandins with 9-anthryldiazomethane (ADAM) (Nimura & Kinoshita, 1980). Since ADAM contains many impurities which interfere with the h.p.l.c. determination, the purification of prostaglandins esterified with ADAM (PGs-ADAM) was attempted by use of a normal-phase silica cartridge (Sep-pak, Waters Associates). The cartridge was prepared by rinsing it with 5 ml of methanol followed by 10 ml of benzene-ethyl acetate (60:40). The sample was passed through the cartridge. The cartridge was washed with benzene-ethyl acetate (60:40, 7 ml) and the PGs-ADAM was then quantitatively eluted with benzene-ethyl acetate-methanol (60:40:5, 7 ml). Peak heights were measured for the quantification of the PGs-ADAM relative to the standard derivatives prepared from authentic PGE_2 and $PGF_{2\alpha}$.

Results are presented as mean ± s.e.mean. Statistical significance was calculated by Student's paired *t* test.

Results Figure 1 illustrates the effects of various concentrations of YS980 on PGE_2 and $PGF_{2\alpha}$ synthesis in rabbit kidney medulla slices. Medulla slice preparations under basal conditions, without the addition of YS980, produce $PGF_{2\alpha}/PGE_2$ in a ratio of

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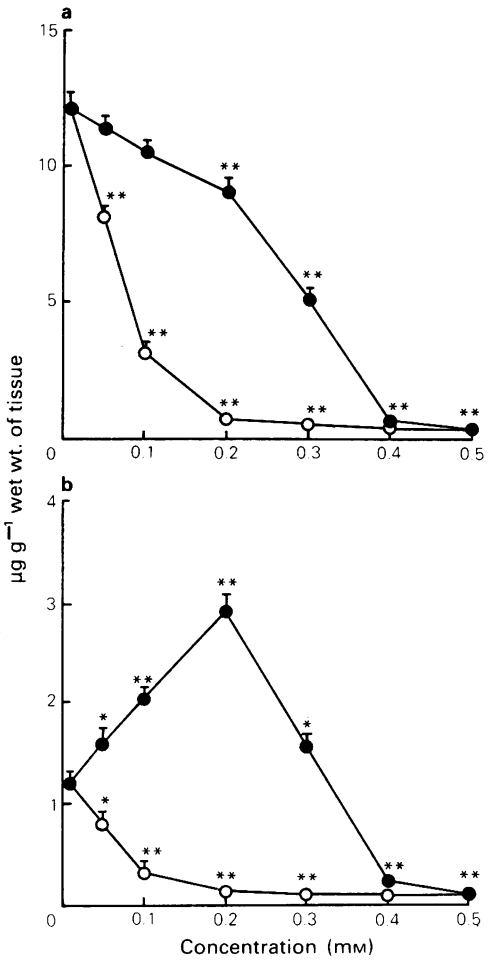

Figure 1 Effect of YS980 on prostaglandin E_2 (a) and prostaglandin $F_{2\alpha}$ (b) synthesis in rabbit kidney medulla slices. Slices were incubated for 30 min at 37°C in 0.15 M KCl/0.02 M Tris-HCl buffer in the presence of different concentrations of YS980 (●) or aspirin (○). Each point indicates the mean of 5 experiments; vertical lines show s.e.mean. $*P < 0.05$ compared with the corresponding value in the absence of YS980 or aspirin. $**P < 0.01$ compared with the corresponding value in the absence of YS980 or aspirin.

0.10. The rate of PGE_2 synthesis appears to be significantly higher than $PGF_{2\alpha}$.

YS980 inhibited medullary generation of PGE_2 at concentrations ranging from 0.05 to 0.5 mM. The effect of YS980 was concentration-dependent. On the other hand, the synthesis of $PGF_{2\alpha}$ was increased by YS980 at concentrations of 0.3 mM and below, with a maximum increase of 137% compared with control. At concentrations of YS980 higher than those causing maximal stimulation a reversal of effect was seen, resulting in inhibition of $PGF_{2\alpha}$ formation at 0.4 mM. Thus, YS980 inhibited markedly both PGE_2 and $PGF_{2\alpha}$ formation at concentrations of 0.4 mM or more (PGE_2, 94–97% inhibition; $PGF_{2\alpha}$, 83–89% inhibition). The effect of YS980 (0.2 or 0.4 mM) was apparent within 10 min after addition to the incubation mixture and persisted for 30 min (data not shown).

The effect of YS980 on prostaglandin synthesis in medulla slices was compared to the effect of aspirin, a representative cyclo-oxygenase inhibitor (Vane, 19791). Aspirin (0.05–0.5 mM) reduced the production of basal PGE_2 and $PGF_{2\alpha}$ in a dose-dependent manner (Figure 1). Maximal inhibition was observed with 0.2 mM aspirin. The inhibitory effect of 0.4 mM YS980 on PGE_2 and $PGF_{2\alpha}$ synthesis was approximately comparable to that of 0.2 mM aspirin.

Discussion The conversion of arachidonate to PGE_2 or $PGF_{2\alpha}$ may be separated essentially into two components. Firstly, PGH_2 synthetase catalyses the oxygenation of arachidonate to PGG_2 (cyclo-oxygenase) and the subsequent reduction of PGG_2 to PGH_2 (peroxidase) (Nugteren & Hazelhof, 1973; Hamberg *et al.*, 1974; Miyamoto *et al.*, 1976; Van der Ouderaa *et al.*, 1977). Secondly, an endoperoxide E_2 isomerase catalyses rearrangement of PGH_2 into PGE_2, or an endoperoxide reductase catalyses reduction of PGH_2 into $PGF_{2\alpha}$ (Hamberg & Samuelsson, 1973).

The present study showed that the inhibition of PGE_2 synthesis by YS980 in concentrations below 0.2 mM was accompanied by a simultaneous increase in the amount of $PGF_{2\alpha}$. It seems possible that YS980 has a distinct effect in promoting the reduction of PGH_2 to $PGF_{2\alpha}$. YS980 at 0.4 mM inhibited both PGE_2 and $PGF_{2\alpha}$ synthesis to the same extent as 0.2 mM aspirin. YS980 at a high concentration therefore seems to be a potent inhibitor of the initial fatty acid oxygenation.

PGE_2 and $PGF_{2\alpha}$ often possess opposite effects within the body (Flower, 1974), suggesting that some pharmacological action of YS980 may be related to its ability to modulate prostaglandin synthesis. The mechanisms of modulation remain to be investigated, however, we have provided the first direct evidence that YS980, an angiotensin-converting enzyme inhibitor, has the potential to modulate PGE_2 and $PGF_{2\alpha}$ synthesis in different ways depending on the concentration.

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