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Effect of tiopronin on prostaglandin synthesis in rabbit kidney medulla slices

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Abstract—The effect of 2-mercaptopropionylglycine (tiopronin), which is widely used for the treatment of various hepatic disorders, on the generation of medullary prostaglandins (PG) E_2 and $F_{2\alpha}$ has been examined. Tiopronin had a potent inhibitory effect on PG E_2 formation. Simultaneously, PG $F_{2\alpha}$ production was increased. In the presence of tiopronin the net increased amount of PG $F_{2\alpha}$ was much smaller than the net decreased amount of PG E_2 (6–20%). These results suggest that tiopronin has the potential to modulate PG E_2 and $F_{2\alpha}$ synthesis by affecting endoperoxide E_2 isomerase or endoperoxide reductase and that this effect may represent some pharmacological action of the drug.

It has been reported that 2-mercaptopropionylglycine (tiopronin), a sulphhydryl compound, reduces the hepatotoxicity of paracetamol or carbon tetrachloride (Labadarios et al 1977; Horiuchi et al 1979). Thus, it is widely used for the treatment of various hepatic disorders. The clinical course of patients with liver cirrhosis is frequently complicated by progressive impairment of renal sodium handling (Epstein 1979). The renal medulla is rich in prostaglandins (PGs) as well as in the enzymes that biosynthesize them. Intrarenal PGs, seem to be determinants of renal haemodynamics and renal sodium handling in both normal and cirrhotic man (Epstein et al 1982). Recently, we have reported that sulphhydryl compounds, such as reduced glutathione and cysteine, play a role in the control of PG E_2 and $F_{2\alpha}$ synthesis in renal medulla (Fujita et al 1986). Those findings prompted us to examine the effect of tiopronin on the in-vitro generation of medullary PGE_2 and $F_{2\alpha}$.

Materials and methods

Male rabbits (2–2·5 kg wt.) were used. The kidneys were removed from anaesthetized (sodium pentobarbitone, 30 mg kg^{-1}) rabbits and rapidly chilled in ice-cold 0·9% NaCl (saline). The kidney medulla slices were prepared as previously described (Fujimoto & Fujita 1982). In all experiments, the slices (0·4 g) were preincubated in 4·0 mL 0·15 M KCl/0·02 M Tris HCl buffer (pH 7·4) at 4°C for 5 min. After preincubation, the medium was discarded, the slices rinsed twice with the Tris HCl buffer and incubated with the indicated concentrations of tiopronin (Santen Pharmaceuticals Ltd, Japan) at 37°C for 30 min.

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We reported previously that the major PGs produced in our incubation of medulla slices and recovered in the medium were E_2 and $F_{2\alpha}$ (Fujimoto et al 1983). PG E_2 and $F_{2\alpha}$ in the incubation medium were simultaneously determined by a high-pressure liquid chromatographic (HPLC) method as described by Fujita et al (1986). Briefly, PG E_2 and $F_{2\alpha}$ extracted with ethyl ether (approximately pH 3) were measured after esterification of the PGs with 9-anthryldiazomethane (ADAM) (Nimura & Kinoshita 1980). Since ADAM contains many impurities which interfere with the HPLC determination, the purification of PGs esterified with ADAM (PGs-ADAM) was attempted using 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 v/v). The sample was passed through the cartridge. The cartridge was washed with benzene–ethyl acetate (60:40 v/v, 7 mL) and the PGs-ADAM was then quantitatively eluted with benzene–ethyl acetate–methanol (60:40:5 v/v, 7 mL). Peak heights were measured for the quantification of the PGs-ADAM relative to the standard derivatives prepared from authentic PG E_2 and $F_{2\alpha}$.

The values presented herein are the means ± s.e.m. Statistical significance was calculated using Student's paired *t*-test.

Results

Fig. 1 illustrates the effects of various concentrations of

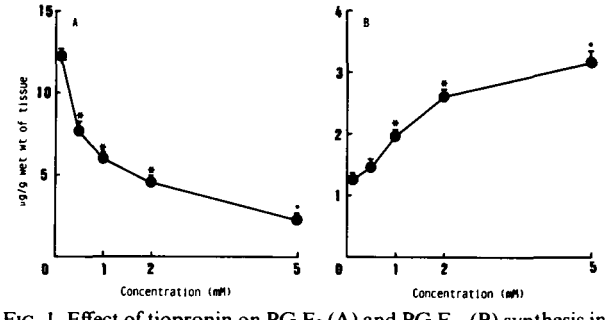

FIG. 1. Effect of tiopronin on PG E_2 (A) and PG $F_{2\alpha}$ (B) synthesis in rabbit kidney medulla slices. Slices were incubated for 30 min at 37°C in 0·15 M KCl/0·02 M Tris HCl buffer in the presence of different concentrations of tiopronin. Each point indicates the mean of 5 experiments; vertical lines show s.e.m. $*P < 0·01$ compared with the corresponding value in the absence of tiopronin.

tiopronin on PG E_2 and $F_{2\alpha}$ synthesis in rabbit kidney medulla slices. The preparation under basal conditions, without the addition of tiopronin, produces PG $F_{2\alpha}/E_2$ in a ratio of 0·10. The rate of PG E_2 synthesis appears to be significantly higher than that of PG $F_{2\alpha}$.

Tiopronin, at concentrations ranging from 0·5 to 5 mM, reduced the production of basal PG E_2 (Fig. 1A). The effect was concentration-dependent. On the other hand, tiopronin at four concentrations stimulated the generation of PG $F_{2\alpha}$ in a dose-dependent manner (Fig. 1B). The effect of tiopronin (1 mM) was apparent within 10 min after addition to the incubation mixture and persisted for 30 min (Fig. 2).

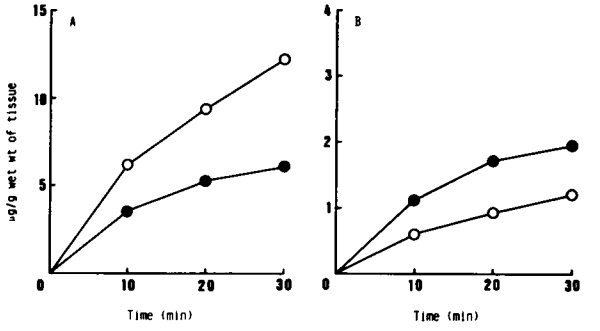

FIG. 2. Time course of PG E_2 (A) and PG $F_{2\alpha}$ (B) release from rabbit kidney medulla slices. Incubations were for 30 min at 37°C in 0·15 M KCl/0·02 M Tris HCl buffer in the absence (○) and the presence of 1 mM tiopronin (●). Each point indicates the mean of 5 experiments (s.e.m. values were less than 5%).

Discussion

The conversion of arachidonate to PG E_2 or $F_{2\alpha}$ may be separated essentially into two components. Firstly, prostaglandin endoperoxide synthetase (cyclooxygenase) catalyses the oxygenation of arachidonate to prostaglandin G_2 and the subsequent reduction of PG G_2 to PG H_2 (Nugteren & Hazelhof 1973; Hamberg et al 1974; Miyamoto et al 1976; Van der Ouderaa et al 1977; Ogino et al 1978). Secondly, an endoperoxide E_2 isomerase catalyses rearrangement of PG H_2 into PG E_2, or an endoperoxide reductase catalyses reduction of PG H_2 into PG $F_{2\alpha}$ (Hamberg & Samuelsson 1973). Another enzyme of potential importance in metabolic interconversion of PG E_2 and $F_{2\alpha}$ in the kidney is PG E_2-9-ketoreductase. With regard to the contribution of PG E_2-9-ketoreductase to control of intrarenal levels of PG E_2 and $F_{2\alpha}$, to date the available evidence is inconclusive (Cagen & Baer 1987). However, comparison of isotope ratios after incubation of rabbit renal medullary slices with a mixture of ^{14}C- and ^{3}H-labelled arachidonic acid indicated that PG $F_{2\alpha}$ was formed in this tissue by reduction of PG H_2 and not by reduction of PG E_2 (Qureshi & Cagen 1982).

In the present study, tiopronin had a potent inhibitory effect on PG E_2 formation. Simultaneously, tiopronin was capable of stimulating PG $F_{2\alpha}$ generation. In the presence of tiopronin the net increased amount of PG $F_{2\alpha}$ was much smaller than the net decreased amount of PG E_2 (6–20%) (Fig. 1). If tiopronin, which selectively stimulates PG $F_{2\alpha}$ biosynthesis, does so by the non-enzymatic reduction of PG H_2, it is difficult to understand why the ratio of net increased PG $F_{2\alpha}$ formation to net decreased PG E_2 formation is not 1 : 1. It is possible that tiopronin participates in a process which leads to inactivation of endoperoxide E_2 isomerase with a concomitant activation of endoperoxide $F_{2\alpha}$ reductase in rabbit kidney medulla slices.

PG E_2 and $F_{2\alpha}$ often possess opposite effects within the body (Flower 1974), suggesting that some pharmacological action of tiopronin may be related to its ability to modulate prostaglandin synthesis. Further studies are needed to clarify the mechanism of modulation; however, we have provided the first direct evidence that tiopronin, which is widely used for the treatment of various hepatic disorders, has the potential to modulate PG E_2 and $F_{2\alpha}$ synthesis by the kidney.

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