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PROSTAGLANDIN BIOSYNTHESIS IN RABBIT KIDNEY: MEPACRINE INHIBITS RENOMEDULLARY CYCLOOXYGENASE*

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Abstract—Mepacrine was found to exert a dose-dependent inhibition of prostaglandin E_2 synthesis in rabbit kidney medulla slices and in medullary microsomes. Mepacrine at 0.5 mM produced 90% inhibition of microsomal prostaglandin E_2 biosynthesis from added arachidonic acid. This effect results from inhibition of medullary cyclooxygenase; the activities of the prostaglandin G_2 hydroperoxidase and the prostaglandin H_2 isomerases are unaffected. In experiments with medulla slices prelabelled with [^{14}C]arachidonate, the effect of mepacrine on the inhibition of [^{14}C]prostaglandin generation was significantly higher (2.5 to 3.5-fold) than its inhibition of [^{14}C]arachidonate release. Hence, although mepacrine reduces prostaglandin production by decreasing the lipolytic release of arachidonate from medullary lipids, its inhibitory effect on prostaglandin cyclooxygenase activity is substantial and appears to contribute significantly to its overall inhibition of prostaglandin generation in kidney medulla. Mepacrine is thus not only a non-selective antilipolytic agent but also a potent cyclooxygenase inhibitor.

Mepacrine was shown to inhibit the release of arachidonic acid and the generation of its oxygenated products in blood platelets [1], rat kidney [2] and the perfused guinea-pig lung [3–5]. Several reports [3, 5] suggested that the drug exerts its effect by inhibiting phospholipase A_2 action, limiting arachidonate hydrolysis from phospholipids and thereby its availability as a precursor for prostaglandins. In support of this, Vigo and co-workers [6] have shown that mepacrine directly inhibits the activities of snake venom and porcine pancreas phospholipase A_2. Furthermore, Hofmann *et al.* [7] have recently shown that mepacrine also inhibits phosphatidylinositol-specific phospholipase C in platelets.

Recent interest in the relationship between arachidonic acid release, generation of oxygenated products and other cellular functions has led to the use of mepacrine to block arachidonate product formation by inhibition of arachidonate release [8, 9]. We, in the course of our studies on lipolysis and prostaglandin bisynthesis in rabbit kidney, have also employed mepacrine in order to study the properties of esterified arachidonate hydrolysing activities, distinct from arachidonate oxygenation reactions. We found, however, that mepacrine also directly inhibits the conversion of arachidonic acid into prostaglandins. After the completion of these studies, Abdel-Latif and Smith [10] reported results on the effect of mepacrine on rabbit iris prostaglandin generation which are in part similar to ours.

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MATERIALS AND METHODS

Preparation of medulla slices and subcellular fractions. Kidneys from rabbits (male, 2.5–3.0 kg) were removed and medulla slices prepared as described [11]. In some experiments, medulla slices were homogenized in Tris–sucrose bufffer (0.3 M sucrose, 5 mM Tris–HCl, pH 8.0) and subcellular fractions (crude plasma membrane, mitochondria, microsomes) were prepared [12].

Incorporation of radioactive arachidonic acid into medullary lipids. Medulla slices (5–7 g) were incubated in 10 ml Tris–HCl buffer (0.1 M, pH 8.0) with 7.0 μCi [1–^{14}C]arachidonic acid for 20 min at 37° with shaking. Following incubation the medium was discarded and the slices were rinsed twice with 0.1 M Tris–HCl buffer (pH 8.0) containing bovine serum albumin (2 mg/ml).

Experiments with medulla slices and microsomes. Medulla slices (0.2–0.3 g) prelabelled with [^{14}C]arachidonic acid were preincubated in 2 ml 0.1 M Tris–HCl buffer (pH 8.0) for 20 min at 37° with shaking in the absence or presence of mepacrine. Following preincubation, the medium was analysed for radioactive arachidonic acid metabolites.

Medulla microsomes were incubated with [^{14}C]arachidonic acid (added as a sodium salt in Tris buffer, pH 8.0) in 1 ml Tris–HCl buffer containing hydroquinone (5 μM), L-epinephrine (1 mM) and tryptophan (1 mM). The incubations were carried out (37°, 20 min, open air tubes with shaking) in the presence of different concentrations of mepacrine. The incubations were terminated by the additon of chloroform–methanol, the lipids extracted and radioactive arachidonate metabolites quantitated by

thin-layer chromatography (TLC) and liquid scintillation.

Assay of radioactive arachidonate products. To media from experiments with medulla slices or total incubation media from experiments with medulla microsomes, 20 volumes of chloroform–methanol (2:1) were added. The extracts were washed sequentially with 4 ml 0.05 N H_2SO_4 and 3.5 ml of distilled water, dried over Na_2SO_4 and concentrated *in vacuo*. The extracts were separated by TLC (ethyl acetate–isooctane–acetic acid–water, 110:50:20:100, by volume, upper phase), radiochromatogram scans obtained and the zones corresponding to authentic prostaglandins E_2, $F_{2\alpha}$ and D_2 and arachidonic acid were scraped and counted by liquid scintillation spectrometry.

Materials. Prostaglandin H_2 was prepared and purified as described previously [13]. Prostaglandins E_2, D_2, $F_{2\alpha}$ and A_2 were kindly provided by Dr. U. Axen and Dr. J. E. Pike of Upjohn Co. (Kalamazoo, MI). [1-^{14}C]Arachidonic acid (specific activity 55 Ci/mole) was obtained from the Radiochemical Centre (Amersham, Bucks, U.K.). Arachidonic acid was obtained from Nu-check (Elysian, MN). L-Epinephrine and mepacrine (quinacrine) were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade.

RESULTS

Mepacrine inhibition of microsomal prostaglandin synthase of kidney medulla

Evidence for the direct inhibitory effect of mepacrine on medullary prostaglandin synthase was obtained from experiments with medullary microsomes incubated with added [^{14}C]arachidonate in the presence of mepacrine. Incubations were performed in Tris buffer supplemented with a mixture of compounds (hydroquinone, epinephrine, tryptophan) previously shown to stimulate overall conversion of arachidonic acid into prostaglandins by microsomes of rabbit medulla [14–16] and other tissues. Mepacrine at 0.5 mM was found to inhibit the biosynthesis of prostaglandins E_2, $F_{2\alpha}$ and D_2 by approximately 89–93% (Fig. 1). In the absence of the added cofactors, prostaglandin biosynthesis was considerably lower (one-third to one-fifth of that indicated in Fig. 1) but the extent of inhibition by mepacrine was approximately the same (87–90%, data not shown). In separate experiments, mepacrine inhibitory potency of PGE_2 formation was measured at several drug concentrations (Fig. 2). Mepacrine was without significant effect at concentrations of 0.1 mM or lower; at 0.5 mM it produced over 90% inhibition. Fron the results shown in Fig. 2, we estimate the IC_{50} of mepacrine for inhibition of renal medullary prostaglandin synthase to be 0.3–0.4 mM.

The biosynthetic pathway for arachidonate conversion to prostaglandins includes three distinct enzymatic steps: cyclooxygenase conversion of arachidonate to the prostaglandin endoperoxide G_2; a peroxidase reduction of prostaglandin G_2 to prostaglandin H_2; and conversion of prostaglandin H_2 to specific prostaglandin products by prostaglandin H_2 metabolizing enzymes. In the normal rabbit kidney, the major prostaglandin H_2 metabolizing activities

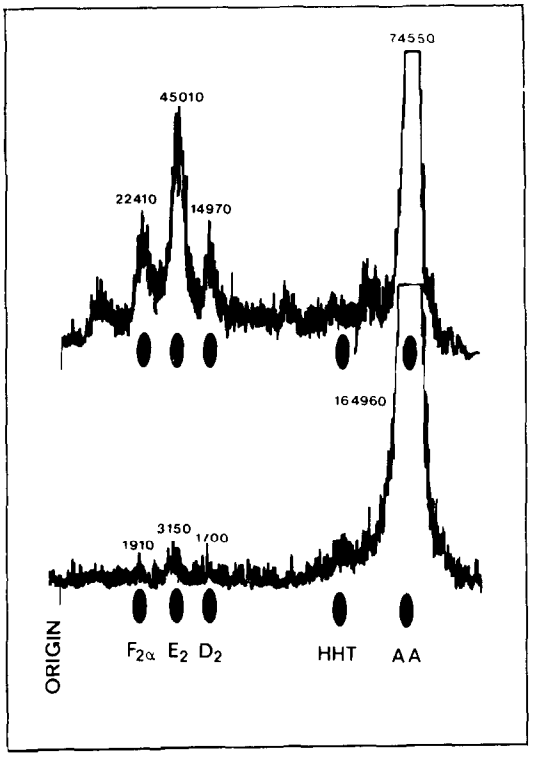

Fig. 1. Mepacrine inhibition of arachidonate conversion to various prostaglandins. Medulla microsomes were incubated (1.5 mg protein in 1.0 ml Tris–HCl buffer, pH 8.0) with arachidonic acid (1 μg, 2×10^5 cpm) in the absence (top panel) or presence (bottom panel) of mepacrine (0.5 mM). A mixture of cofactors (hydroquinone, 5 μM; L-epinephrine, 1 mM; L-tryptophan, 1 mM) was added prior to arachidonic acid. Incubations were carried out for 5 min, the reaction was stopped by the addition of 20 ml chloroform–methanol (2:1) and the extract concentrated and chromatographed. Data shown are the radiochromatogram scans of the TLC plates. Numbers above the peaks are the cpm obtained by scraping and liquid scintillation counting. Results are from one experiment. Similar results were obtained in three similar experiments. Abbreviations used: HHT, 12-L-hydroxy-5,8,10-heptadecatrienoic acid; AA, arachidonic acid.

include isomerases which produce prostaglandins E_2 and D_2, a putative reductase to yield prostaglandin $F_{2\alpha}$ and a prostacyclin synthase. The data in Fig. 1 show that mepacrine had no effect on the relative profile of prostaglandin products obtained, inhibiting to a similar extent the formation of prostaglandins E_2, D_2 and $F_{2\alpha}$. These data thus indicate that mepacrine either: (1) affects similarly the three different prostaglandin H_2 metabolizing activities; or (2) causes no apparent inhibition of specific prostaglandin H_2 metabolizing enzymes and its effect is likely exerted at a preceding enzymic step. The data in Fig. 1 also suggest that mepacrine does not inhibit the prostaglandin G_2 hydroperoxidase since such inhibition would lead to the accumulation of prostaglandin G_2 which undergoes chemical decomposition to prostaglandins E_2 or D_2 and also to 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) [17, 18]. Lack of increased formation of the latter

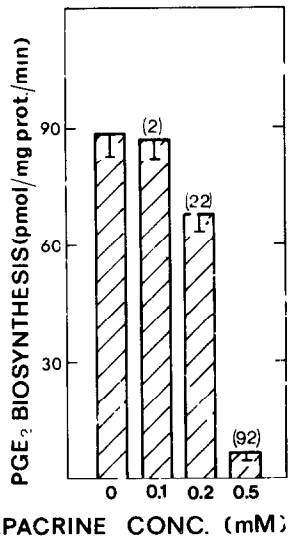

Fig. 2. Mepacrine inhibition of [^{14}C]arachidonate conversion to [^{14}C]prostaglandin E_2 by medulla microsomes. Microsomes (1.5 mg protein in 1 ml Tris buffer) were incubated with arachidonic acid (1.5 μg, 50,000 cpm) for 6 min at 37° in the presence of different concentrations of mepacrine and the amounts of prostaglandin E_2 generated were determined. Results are means ± S.E.M. of three experiments. Numbers in parentheses indicate percentage inhibition by mepacrine.

compound in the presence of mepacrine strongly suggests that the prostaglandin G_2 peroxidase reaction, as well as the prostaglandin H_2 metabolizing activities, is unaffected by this drug. Direct support for this tentative conclusion was obtained from studies in which prostaglandin H_2 was added to kidney microsomes in the presence of 5 mM reduced glutathione. Microsomes preincubated in the presence or absence of 0.5 mM mepacrine produced virtually identical profiles of products (PGE_2, 58–61%; PGD_2, 13–16%; $PGF_{2\alpha}$, 3–5%; HHT, 4–6%). We therefore conclude that mepacrine decreases arachidonate conversion to prostaglandins by inhibition of the cyclooxygenase.

Mepacrine inhibition of acylhydrolases vs prostaglandin synthase activities in medulla slices

Mepacrine inhibition of prostaglandin biosynthesis from esterified arachidonate in medulla slices may thus be a composite effect which includes inhibition of acylhydrolases, thus decreasing the substrate availability of precursor arachidonate and the inhibition of prostaglandin synthase. An antilipolytic effect of mepacrine was indeed suggested to account for its inhibition of prostaglandins and other arachidonate product generation [3, 5]. The possible contribution of cyclooxygenase inhibition to the overall inhibition was, however, less appreciated.

In order to measure simultaneously the effects of mepacrine on both arachidonate release and prostaglandin biosynthesis, we prepared medulla slices prelabelled with [^{14}C]arachidonic acid, incubated them with mepacrine and determined the drug's effect on both the net release of [^{14}C]arachidonic acid and the generation of [^{14}C]prostaglandin products. Serum albumin was not included in the incubation medium in order to avoid extracellular binding and thus enhance basal release of free arachidonate. The observed release is therefore an apparent net rate which reflects both release and partial reacylation. Mepacrine at a concentration of 0.5 mM caused only a marginal (approximately 9%) reduction in the release of [^{14}C]arachidonic acid into the medium, whereas the decrease in the synthesis of prostaglandins $F_{2\alpha}$ and E_2 was substantial at 23 and 34%, respectively (Fig. 3). The addition of aspirin (1 mM) to the slices during prelabelling with [^{14}C]arachidonate blocked subsequent prostaglandin formation by 85–89% and quantitatively increased the radioactivity of free arachidonate in the medium. Under these conditions, mepacrine reduced arachidonic acid release by only 8.8–10.5% (range of four experiments). The results thus show that in kidney medulla mepacrine exerts a significant inhibitory effect on prostaglandin synthase, which contributes a substantial part to the overall inhibition by this compound of prostglandin generation from initially esterified arachidonic acid.

DISCUSSION

A number of studies [2–5, 8, 9] have suggested that mepacrine inhibition of arachidonate product generation is due to the inhibition of cellular phospholipase A_2, thereby limiting the availability of substrate arachidonate for subsequent conversion to oxygenated products. Recent studies have shown that mepacrine inhibits the activity of purified phospholipase A_2 from snake venom and porcine pancreas [6]. Interestingly, Hofmann *et al.* [7] have

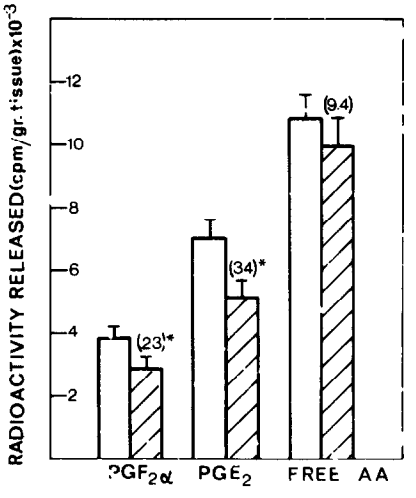

Fig. 3. Inhibition of radioactive prostaglandin generation and arachidonate release in slices treated with mepacrine. Slices prelabelled with [^{14}C]arachidonic acid were incubated in the absence (open bars) or presence (hatched bars) of 0.5 mM mepacrine for 30 min at 37°. Following incubation, the media were analysed for radioactive prostaglandins and arachidonic acid. Values given are means ± S.E.M. of four experiments. Numbers in parentheses indicate percentage inhibition by mepacrine. The asterisk (*) indicates statistical significance ($P < 0.05$, t-test). Abbreviations: PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; AA, arachidonic acid.

recently shown that mepacrine also inhibits phosphatidylinositol-specific phospholipase C in human platelets, concluding that the compound is not a selective inhibitor for phospholipase A_2.

The data presented here show that mepacrine is also a potent inhibitor of microsomal prostaglandin synthase from kidney medulla (Figs. 1 and 2). Mepacrine at concentrations of 0.1 mM or lower had no effect on prostaglandin production, whereas almost complete inhibition (92%) was obtained at 0.5 mM (Fig. 2). Abdel-Latif and Smith [10] have recently reported on the effect of mepacrine on prostaglandin synthase activity in the rabbit iris smooth muscle and iris microsomes. They observed a biphasic effect of mepacrine: a significant increase (up to 110%) in the synthesis of prostaglandins E_2 and $F_{2\alpha}$ at low mepacrine concentrations (0.1 mM and below), but a significant inhibition at higher concentrations. The reason for the difference between our results and those of Abdel-Latif and Smith is not clear, but may relate to tissue differences between the iris and the kidney.

Our results also show that mepacrine equally inhibits the formation of prostaglandins E_2, D_2 and $F_{2\alpha}$ and does not stimulate the formation of 12-L-hydroxy-5,8,10-heptadecatrienoic acid (Fig. 1). These results, together with the fact that mepacrine did not inhibit any of the prostaglandin H_2 metabolizing enzymes, led us to conclude that mepacrine inhibits the cyclooxygenase component of the prostaglandin synthase system. Recent work by Sinha [19] showed that mepacrine is reacted upon by ram seminal vesicle microsomes to yield a free radical. Such a reaction or the resulting free radical may inhibit arachidonate oxygenation by the cyclooxygenase. Mepacrine may also inhibit the cyclooxygenase enzyme indirectly via interaction with membrane phospholipids, most notably phosphatidylethanolamine, as reported recently in platelets [20], thereby causing perturbation of the microsomal membrane.

In medulla slices, the mepacrine inhibiting potency of the cyclooxygenase substantially exceeded its apparent inhibition of basal medullary acylhydrolase activity. At 0.5 mM, mepacrine inhibited the overall conversion of esterified arachidonate to prostaglandin E_2 by 35% (Fig. 3) whereas it only marginally reduced the release of radioactive arachidonic acid (Fig. 3), thus indicating that a major inhibitory effect of mepacrine on the overall endogenous prostaglandin biosynthesis is via inhibition of the cyclooxygenase. The fact that mepacrine can affect arachidonate metabolism by several different mechanisms and at separate metabolic steps is a major limitation on its use as a specific experimental tool in studies on arachidonate metabolic pathways.

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