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Reduction of urinary excretion of PGE_2 and 6 keto $PGF_{1\alpha}$ by tiaprofenic acid

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1 Tiaprofenic acid (Surgam, Cassenne) was administered intravenously to saline-diuretic conscious rats, at doses of 2, 10 and 25 mg kg^{-1} body weight.

2 Tiaprofenic acid significantly reduced urinary prostaglandin E_2 (PGE_2) and 6 keto $PGF_{1\alpha}$ excretion, at all three doses employed. The extent of the reduction was similar for both PGE_2 and 6 keto $PGF_{1\alpha}$ output; hence no evidence of 'selectivity' (i.e. sparing of PGI_2 synthesis) was observed.

3 Tiaprofenic acid was also administered to rats receiving an infusion of 5% dextrose. The dose employed (0.5 or 1 mg kg^{-1} body weight) was submaximal and elicited reductions in PGE_2 output to values still more than 60% of the control period values. In this group of animals, the percentage change in 6 keto $PGF_{1\alpha}$ excretion was again not significantly different from that of PGE_2.

4 The maximal extent of reduction in urinary PGE_2 excretion with tiaprofenic acid (25 mg kg^{-1} body weight) was not significantly different from that elicited by indomethacin (10 mg kg^{-1} body weight), although the time course of the reduction was different.

Introduction

The kidneys synthesize prostaglandin I_2, E_2, $F_{2\alpha}$ and D_2, as well as thromboxane A_2 (TXA_2) but the relative importance of these different prostanoids within the normal kidney is still not clear (see, Lote, 1982). PGI_2 is likely to be the primary prostaglandin involved in maintaining renal blood flow and glomerular filtration rate in the presence of vasoconstrictor stimuli (Terragno *et al.*, 1977; Baer & McGiff, 1979); hence, non-steroidal anti-inflammatory drugs (NSAIDS) must be used with caution in patients with impaired renal function. However, patients with chronic glomerular disease have a reduced urinary output of 6 keto $PGF_{1\alpha}$ (the stable metabolite of PGI_2), whereas the output of PGE_2 is unchanged (Ciabattoni *et al.*, 1984). Thus, a drug that 'spared' PGI_2 synthesis could have advantages in patients with impaired renal function.

Tiaprofenic acid (Surgam, Cassenne) is a non-steroidal anti-inflammatory drug derived from propionic acid. Tiaprofenic acid inhibits prostaglandin synthesis (Deraedt *et al.*, 1980) but *in vitro* studies have suggested that the drug may be less potent than other NSAIDS as an inhibitor of prostacyclin (PGI_2) synthesis (Shror *et al.*, 1980).

In the present experiments the urinary excretion in conscious rats of PGE_2 and 6 keto $PGF_{1\alpha}$ (the stable metabolite of PGI_2) has been measured by radioimmunoassay, following the administration of either tiaprofenic acid or indomethacin.

Methods

Experiments were performed on male rats (weight range 250–400 g, Sprague-Dawley strain) which were allowed free access to food and water prior to the experimental day.

On the experimental day, each rat was lightly anaesthetized with ether, and a flexible cannula (Portex PP25) was implanted in a lateral tail vein. The animals were then placed in individual Perspex restraining cages and allowed to regain consciousness. An infusion was then begun, via the tail cannula. Six groups of animals were used, denoted A, B, C, D, E, F. Groups A–D received a saline (0.9%, 5.8 ml h^{-1}) infusion, whereas groups E and F received a dextrose (5%, 5.8 ml h^{-1}) infusion.

At 3 h 52.5 min the animals received, incorporated in the infusate, over a 15 min period, the following: Group A ($n = 9$): indomethacin 10 mg kg^{-1} body weight; Group B ($n = 6$): tiaprofenic acid 2 mg kg^{-1} body weight; Group C ($n = 8$): tiaprofenic acid 10 mg kg^{-1} body weight; Group D ($n = 6$): tiaprofenic acid 25 mg kg^{-1} body weight; Group E ($n = 5$):

tiaprofenic acid 1.0 mg kg^{-1} body weight and Group F ($n = 4$): tiaprofenic acid 0.5 mg kg^{-1} body weight.

Urine was obtained from each rat at 2 h and thereafter at hourly intervals throughout the infusion. The samples were collected into plastic vials and were cooled to −20°C immediately after collection. They were assayed for prostaglandins (PGE_2 and 6 keto $PGF_{1\alpha}$) within 4 weeks.

Prostaglandin radioimmunoassay

Aliquots (2 ml) of urine were extracted and assayed. The procedure has been described previously (Lote *et al.*, 1983).

Extraction markers (2.5 nCi [^{3}H]-PGE_2, 120–170 Ci $mmol^{-1}$, or 1.25 nCi [^{3}H]-6 keto $PGF_{1\alpha}$, 120–180 Ci $mmol^{-1}$) were incorporated in the aliquots prior to the start of the extraction procedure. This introduces a small error (< 5%) into the radioimmunoassay, for which no correction was made.

Prior to the radioimmunoassays, E series prostaglandins and 6 keto $PGF_{1\alpha}$ were separated from each other and from other prostaglandins by chromatography on silicic acid columns, using 60–200 mesh silicic acid (SIL-A-200, Sigma Chemical Company) 0.25 g, prepared by suspending the silicic acid in 4 ml toluene : ethyl acetate (6:4 by vol). The columns were washed with 2 × 2 ml methanol, then 2 × 2 ml toluene : ethyl acetate (6:4). Samples for chromatography, which had previously been evaporated to dryness, were applied to the column in 0.2 ml toluene : ethyl acetate : methanol (60:40:10). Then 4 × 2 ml toluene : ethyl acetate (6:4) was passed through the column to elute A series prostaglandins. E series prostaglandins were eluted with 4 × 2 ml toluene : ethyl acetate : methanol (60:40:4), or (on separate columns), 6 keto $PGF_{1\alpha}$ was eluted with methanol.

For the PGE_2 assay, a prostaglandin E specific antiserum was used (Miles-Yeda, Rehovot, Israel), which has 70% cross-reactivity with PGE_1. However, this cross-reactivity is of little consequence since the kidney does not synthesize PGE_1.

For the 6 keto $PGF_{1\alpha}$ assay, antiserum was obtained from New England Nuclear, Dreieich, FRG.

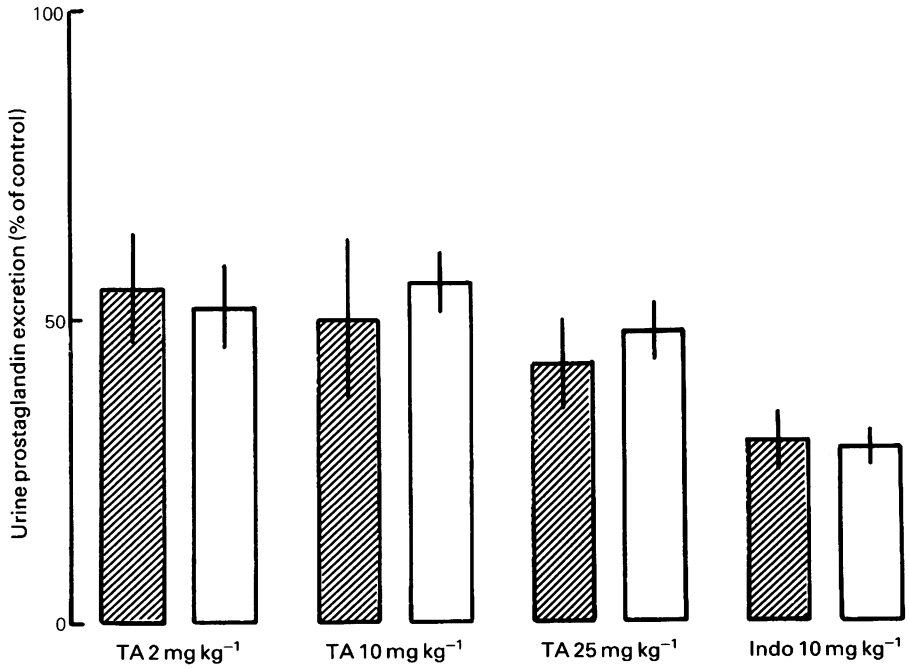

Figure 1 Urinary prostaglandin excretion in the 2 h period following the intravenous administration of tiaprofenic acid (TA) or indomethacin (Indo), at the doses shown. Results are expressed as a percentage of the value in the 2 h period prior to NSAID administration. Hatched bars show PGE_2 excretion, and open bars show 6 keto $PGF_{1\alpha}$ excretion. The reduction in PGE_2 and 6 keto $PGF_{1\alpha}$ excretion, compared to the control period, is significant ($P < 0.01$) for all doses of tiaprofenic acid and for indomethacin. There were no significant differences between the extent of PGE_2 inhibition and that of 6 keto $PGF_{1\alpha}$ in any of the groups.

The extraction efficiency was 39.3 ± 1.2% ($n = 107$) for PGE_2, and 42.0 ± 1.1% ($n = 102$) for 6 keto $PGF_{1\alpha}$. To assess the accuracy of our radioimmunoassays, experiments were performed in which urine samples were divided into 12 aliquots. Six of these were extracted, separated and assayed as described above. To the remaining six, known amounts (2.85 pmol ml^{-1} PGE_2, 1.35 pmol ml^{-1} 6 keto $PGF_{1\alpha}$) of prostaglandins were added before the extraction step; the calculated and actual increases in the assayed levels were then compared. The ratio of measured increase/calculated increase was 1.15 ± 0.04 for 6 keto $PGF_{1\alpha}$, and 0.86 ± 0.03 for PGE_2.

Pretreating urine samples with activated charcoal, to remove prostaglandins, followed by radioimmunoassay of these 'stripped' samples, gave results not significantly different from the water blank in the assays.

Statistical methods

Results are expressed as mean ± s.e.mean. The significance of differences within groups was assessed by the paired t test. The significance of differences between groups was assessed by Student's t test.

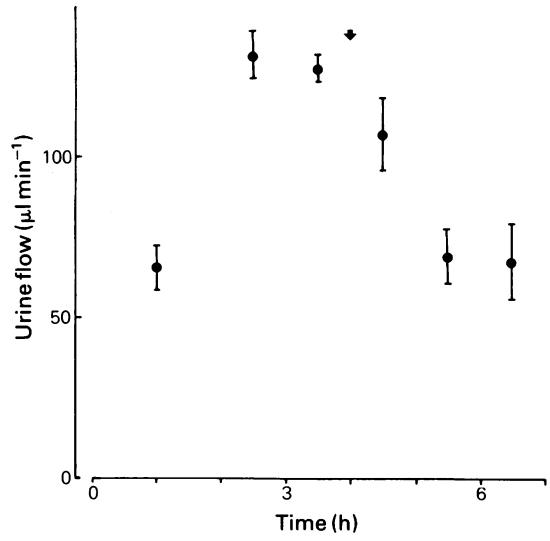

Figure 2 Effect of tiaprofenic acid (25 mg kg^{-1} body weight) on urine flow. Tiaprofenic acid was administered intravenously for 15 min from 3 h 52.5 min to 4 h 7.5 min. The effect on urine flow was maximal in the 5–7 h period.

Results

Saline-infused groups

The control (2–4 h period) output of PGE_2 was 59.7 ± 7.7 pmol $2\,h^{-1}$, and of 6 keto $PGF_{1\alpha}$ was 27.2 ± 3.6 pmol $2\,h^{-1}$ ($n = 29$). Tiaprofenic acid significantly reduced the excretion of both PGE_2 and 6 keto $PGF_{1\alpha}$ (Figure 1). The reductions in excretion of PGE_2 and 6 keto $PGF_{1\alpha}$ were significant at all three doses employed) 2, 10 and 25 mg kg^{-1} body weight). However, the extent of the inhibition was not different for PGE_2 compared to 6 keto $PGF_{1\alpha}$, at any of the doses.

In comparison with indomethacin, tiaprofenic acid appears to be a less potent inhibitor of prostaglandin synthesis, on a weight-for-weight basis (Figure 1). However, this impression is somewhat misleading, since the maximal effect of indomethacin (as judged by decreased urine flow) occurs in the 4–5 h infusion period (see Figure 1 of Haylor & Lote, 1980), whereas the maximal effect of tiaprofenic acid occurs in the 6–7 h period (Figure 2). When the reduction in PGE_2 and 6 keto $PGF_{1\alpha}$ excretion by indomethacin in the 4–6 h period is compared to that by tiaprofenic acid in the 5–7 h period, no differences were apparent. PGE_2 excretion was inhibited by 69 ± 5% by indomethacin (10 mg kg^{-1} body weight) and 70 ± 3% by tiaprofenic acid (25 mg kg^{-1} body weight). The values for 6 keto $PGF_{1\alpha}$ were 70 ± 3% and 69 ± 3% respectively.

Dextrose-infused groups

The control (2–4 h period) outputs of PGE_2 and of 6 keto $PGF_{1\alpha}$ were not significantly different from those obtained during saline infusion. However, at the low doses of tiaprofenic acid used in these groups, individual animals could respond with inhibitions of prostaglandin synthesis as great as those seen at higher doses, or with a smaller degree of inhibition, or with no reduction in PGE_2 and 6-keto $PGF_{1\alpha}$ output at all.

The objective in these low dose experiments was to determine whether there was any evidence of 'selectivity' in the action of tiaprofenic acid, when the dose administered was submaximal. Accordingly, those animals in which tiaprofenic acid elicited a decrease in PGE_2 excretion, in the 5–7 h period, to a value still greater than 60% of the control (2–4 h) value, were regarded as a single group (although in two of these animals the dose of tiaprofenic acid employed was 0.5 mg kg^{-1} body weight, and in the other three animals it was 1 mg kg^{-1} body weight). The inhibitions of 6 keto $PGF_{1\alpha}$ and PGE_2 in the 5–7 h period were compared in this group ($n = 5$). For PGE_2, the 5–7 h PGE_2 output was 77.8 ± 4.9% of the 2–4 h value ($P < 0.02$), whereas for 6 keto $PGF_{1\alpha}$ the figure was 57.9 ± 6.1% ($P < 0.01$). Hence there is no evidence for 'selective' inhibition of PGE_2 rather than 6 keto $PGF_{1\alpha}$. The difference between the percentage inhibitions of the two prostanoids is not significant.

Discussion

It is well known that non-steroidal anti-inflammatory drugs do not completely suppress urinary prostanoid excretion (e.g. Berl *et al.*, 1977) and the maximal percentage inhibition elicited can be variable (Lote *et al.*, 1984). Nevertheless, the results of the present study in saline-infused rats clearly demonstrate that tiaprofenic acid is an effective prostaglandin synthesis inhibitor and that it is approximately equipotent with indomethacin, although this comparison is complicated by different latencies of the inhibitory effect for the two drugs, and may also be affected by factors such as different changes in urine flow (Kirschenbaum & Serros, 1980) or pH (Haylor *et al.*, 1984) with different inhibitors. However, there was no evidence that tiaprofenic acid inhibited PGI_2 synthesis (as measured by 6 keto $PGF_{1\alpha}$ excretion) less than PGE_2 synthesis, at any of the doses employed or that tiaprofenic acid was different from indomethacin in the degree of inhibition of PGI_2 synthesis elicited.

The series of animals in which dextrose was infused (and in which tiaprofenic acid doses of 1 mg kg^{-1} body weight and 0.5 mg kg^{-1} body weight were employed) were to investigate the possibility that 'selectivity' (i.e. differential inhibition of PGE_2 and 6 keto $PGF_{1\alpha}$ excretion) might be present when submaximal inhibitory doses of tiaprofenic acid were employed. This would be difficult to establish with certainty in saline-infused animals, since a prolonged isotonic saline infusion *per se* can reduce urinary PGE_2 excretion whereas dextrose infusion does not have this effect (Lote *et al.*, 1983). No evidence of selectivity was found.

The 2 mg kg^{-1} body weight dose of tiaprofenic acid used in the present animal study approximates the therapeutic dose recommended for man (200 mg tablets, which for a 70 kg adult is 2.86 mg kg^{-1} body weight). Although we are aware of the limitations of extrapolating our findings in the rat to man, nevertheless it seems clear that tiaprofenic acid, by inhibiting renal PGI_2 and PGE_2 synthesis, can have the same adverse effects on renal function as most other NSAIDS, so that it should be used with great caution in patients with impaired renal perfusion and reduced glomerular filtration rate.

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