J. Pharm. Pharmacol. 1982, 34: 648–652
Received March 1, 1982

0022–3573/82/100648–05 $02.50/0

Renal handling of indomethacin and its relationship with the secretory pathway of prostaglandins

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The uptake of prostaglandin E_2, one of the main renal prostaglandins and of *p*-aminohippurate, an indicator of the anion organic transport, by slices of kidney cortex from adult female rats was studied in the presence and in the absence of indomethacin. The drug's inhibitory effect on the uptake of prostaglandin E_2 was observed both after in vivo administration as well as when it was present in the bathing media. The effect was more pronounced when the drug was given in vivo and in addition, was present in the bath. [^{14}C]PAH uptake was inhibited by indomethacin in a dose-related pattern and the kinetic analysis of this effect is indicative of a competitive inhibition. As expected, uptake of PAH by medullary slices was not affected by the presence of either indomethacin of PGE_2. Indomethacin was more potent in inhibiting PGE_2 uptake than PAH uptake.

The renal excretion of several exogenous compounds such as penicillin, *p*-aminohippurate (PAH), probenecid and 2,4-dinitrophenol is achieved through the secretory renal pathway for organic anions (Cross & Taggart 1950; Berndt & Grote 1968; Berner & Kinne 1976) and the uptake of PAH by renal cortical slices has been thoroughly validated as a measurement of the carrier-mediated transport of organic anions by the cells of the renal proximal tubule (Cross & Taggart 1950). It is known that probenecid and other substances transported by this mechanism competitively inhibit PAH transport. The uptake of PAH has also been proposed as an indicator of viability of the kidneys to be transplanted and as an indicator of renal toxicity due to several agents such as carbon tetrachloride or mercuric chloride (Kluwe 1981). Recently Irish (1979) reported that prostaglandins are also transported by this system. The affinity of the carrier for the substance to be secreted, and its concentration, determine the amount transported.

After the observation by Skeith et al (1968) that probenecid doubled the blood levels of radioactivity in patients receiving labelled indomethacin, it was suggested that indomethacin, underwent renal secretion via the same pathway as organic anions. However Yesair et al (1970) observed that a large dose of probenecid given intravenously 3 h after indomethacin (while the indomethacin plasma level was falling), induced a significant rise in indomethacin plasma concentrations. These results indicate a complex interaction between indomethacin and probenecid at the secretory level and perhaps at the absorption site as well.

To eliminate some factors such as variation in blood supply to kidney that have been reported when indomethacin is given and the different affinity of indomethacin and of PAH or probenecid for plasma proteins, it was decided to analyse the role of indomethacin on the secretory mechanism of prostaglandin E_2 and PAH, as evaluated by the effect on the uptake of either PAH or PGE_2 by cortical slices. Under this experimental procedure the effect of blood supply to the tubules and the influence of binding of the studied substances to the plasma proteins are eliminated.

Indomethacin had a more pronounced effect on the renal uptake of PGE_2 than on the uptake of PAH.

METHODS

Under light anaesthesia with ether, female adult rats were decapitated and the kidneys removed. Slices from the cortex (100 to 200 μm thick) obtained with a manual slicer were kept in ice-cold Ringer for mammals and gassed with O_2/CO_2 (95/5%), until the experiment, usually 15 to 20min.

Tritiated prostaglandin E_2 ([^{3}H]PGE_2) uptake in the presence of indomethacin

To assess the effect of indomethacin on the uptake of [^{3}H]PGE_2 (1×10^{-9} M) by the renal tissue, cortical slices were incubated in the absence and in the presence of increasing concentrations of indomethacin in the bathing media (from 10^{-7} to 10^{-4} M). Tissue samples were incubated 1 h at 25 °C, gassed

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with O_2/CO_2 (95/5%) and kept under gentle stirring after which the slices were blotted, dried overnight at 80 °C and weighed. Samples were digested with a commercial solubilizer (NCS, Amersham), 10 ml of a scintillation liquid added and radioactivity determined in a liquid scintillation spectrometer (Packard Tri-Carb Mod. 3255). Adequate corrections for quenching and background were made. Non-specific uptake was determined in the presence of a 100-fold excess of non-radioactive PGE_2.

Ringer solution for incubation had the following composition (mM): NaCl 110; KCl 5; $NaHCO_3$ 25; $CaCl_2$ 1; NaH_2PO_4 1·2; $MgSO_4$ 1·2; sodium acetate 10 and glucose 7; pH 7·4, 290 ± 10 mOsm kg^{-1} H_2O.

[^3H]PGE_2 uptake in rats treated with indomethacin

Indomethacin (10 mg kg^{-1} in ethanol, 20 μg $μl^{-1}$) was administered intraperitoneally to 8 female adult rats. Three doses were given at 8 h intervals, the last dose was injected 1 h before the extirpation of the kidneys. A control group (n = 4) received the vehicle. Renal tissues from control and treated rats were incubated in the presence of [^{3}H]PGE_2 (1×10^{-9} M, final concentration). Slices from the indomethacin-treated rats were divided into three groups two of which had indomethacin in the bath at a final concentration of either 10^{-5} or 10^{-4} M and the third group was incubated without additional indomethacin.

Effect of indomethacin on the [^{14}C]p-aminohippurate ([^{14}C]PAH) uptake

The anion transport system is located in the renal cortex and not in the medulla, so if the effect of indomethacin is achieved through inhibition of anion organic transport, no effect should be observed in medullary slices.

Slices from the whole kidney were obtained. Cortex was separated from medulla by dissection and pieces from cortex and medulla were incubated separately with Ringer containing [^{14}C]PAH (1×10^{-6} M) in the absence and in the presence of increasing concentrations of indomethacin (from 1×10^{-6} to 1×10^{-4} M). A 1000-fold excess of non-radioactive PAH was used to measure non-specific uptake. The effect of PGE_2 on the [^{14}C]PAH uptake was also assessed. Increasing concentrations of PGE_2 (from 1×10^{-6} to 1×10^{-4} M, final concentrations), were tested in the presence of [^{14}C]PAH (1×10^{-6} M). A 1000-fold excess of non-radioactive PAH was used to measure non-specific uptake.

Reagents

Tritium labelled prostaglandin E_2 (sp. act. 117 Ci mM^{-1}), ^{14}C-labelled *p*-aminohippuric acid (sp. act. 47.9 Ci M^{-1}) and scintillation liquid (Aquasol), were purchased from New England Nuclear. Indomethacin and non-radioactive PAH was obtained from Sigma Chemical Co. (St Louis Mo.) and NCS, tissue solubilizer, from Amersham/Searle. Non-radioactive PGE_2 was a generous gift from Dr J. E. Pike (The Upjohn Co. Kalamazoo, Mich.).

RESULTS

Effect of indomethacin on the [^3H]PGE_2 uptake

The presence of indomethacin in the bath reduced the [^{3}H]PGE_2 uptake by the cortical slices from 8354 ± 405 to 2953 = 164 d min^{-1} mg^{-1} dry weight ($P < 0·001$) at the highest concentration tested, i.e. 10^{-4} M of indomethacin (Fig. 1). Non-specific uptake amounted to 17.3% of the total radioactivity. Reduction in uptake followed a dose-dependent pattern. IC50, the concentration of indomethacin required to inhibit by 50% the [^{3}H]PGE_2 uptake was 3.6×10^{-6} M. Since most of the cortical uptake is due to active transport of PGE_2, these results suggest that indomethacin interferes with the carrier by decreasing transport.

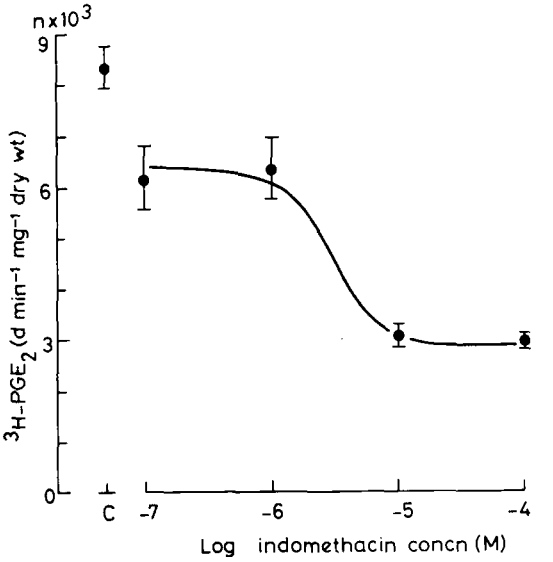

FIG. 1. Inhibitory effect of increasing concentrations of indomethacin in the incubating media on the labelled prostaglandin E_2 uptake by renal cortical slices. Slices were kept at 25 °C, in Ringer containing [^{3}H]PGE_2 at a final concentration of 1×10^{-9} M, equilibrated with O_2/CO_2 (95/5%), for 1 h. Mean ± s.e.m. are shown.

Effect of in vivo *inhibition of prostaglandin synthesis on the* [^{3}H]*PGE$_2$ uptake*

Administration of indomethacin intraperitoneally, previous to preparing slices, decreased [^{3}H]PGE$_2$ uptake from a control value of 7343 ± 394 to 5393 ± 194 d min^{-1} mg^{-1} dry weight ($P < 0{\cdot}001$). The addition of indomethacin (10^{-5} or 10^{-4} M final concentration) to the incubating media further decreased the uptake (4290 ± 183 and 3090 ± 161 d min^{-1} mg^{-1} dry weight, respectively) (Fig. 2).

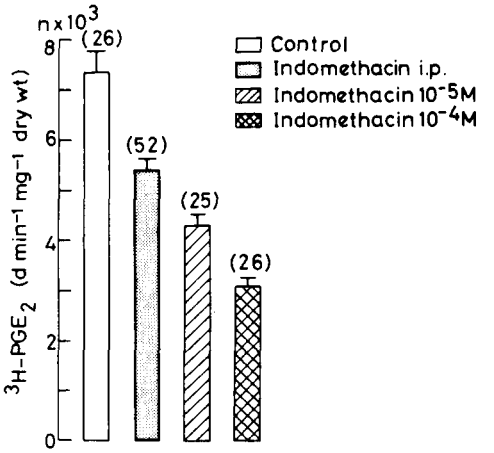

FIG. 2. Effect of indomethacin on the [^{3}H]PGE$_2$ uptake by renal cortical slices. Experiments were carried out in control rats (open column) and in rats treated with indomethacin (10 mg kg^{-1}) i.p., during 24 h before the experiment (stippled column). The effect of additional indomethacin in the bathing media (10^{-5} M (hatched column) and 10^{-4} M (cross hatched column)) was also investigated. Columns show the value of the mean and lines equal s.e.m. (□) = number of slices. The differences from control value are statistically significant ($P < 0{\cdot}001$) in all the indomethacin treated groups.

In spite of a rather high intraperitoneal dose of indomethacin, a more marked inhibition of the uptake was observed when the drug was added to the incubating media, suggesting that higher concentrations are needed to achieve a full inhibitory effect on the prostaglandin uptake.

Inhibitory effect of indomethacin on the [^{14}C]*PAH uptake*

The uptake of PAH by renal cortical slices has been accepted as an adequate model to measure the transport pathway of the organic anions by the renal proximal tubules (Cross & Taggart 1950). When [^{14}C]PAH uptake was measured in the presence of increasing concentrations of indomethacin, an inhibitory effect of indomethacin was observed, suggesting that both compounds share some step of the transporting mechanism. IC50 for indomethacin on the [^{14}C]PAH uptake was $1{\cdot}6 \times 10^{-5}$ M. Non-specific uptake amounted to 29·1% of the total uptake. As expected, no significant difference in the medullary slices was observed at any concentration of indomethacin between control and experimental groups, suggesting that the inhibitory effect observed in the cortex is mainly due to decreased transport. The amount of [^{14}C]PAH in medullary slices is about equal to that observed in the presence of an excess of non-radioactive PAH, indicating that it is due to non-specific uptake (471 ± 14 d min^{-1} mg^{-1} dry weight in the presence of indomethacin 10^{-4} M and 469 ± 33 d min^{-1} mg^{-1} dry weight in the presence of PAH 10^{-3}M) (Fig. 3).

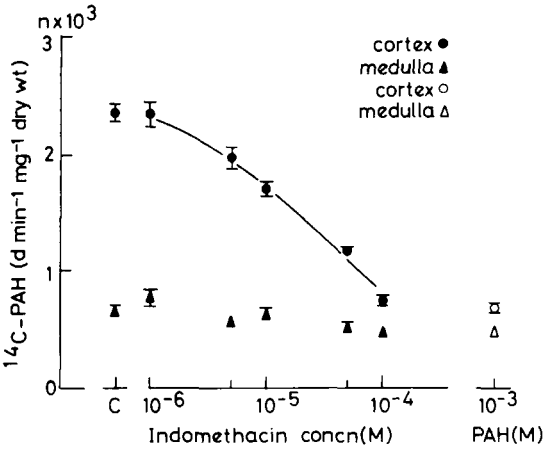

FIG. 3. Inhibition of the [^{14}C]PAH uptake (1×10^{-6} M) by increasing concentrations of indomethacin in renal cortical (● ○) slices from female rats. No effect was observed in medullary (▲ △) slices, suggesting that the inhibitory effect of indomethacin is mainly due to a decrease in the carrier mediated transport of PAH. Symbols represent mean ± s.e.m. In those points where there is no indication of this last value, it is included within the symbol.

The effect of PGE$_2$ on the uptake of [^{14}C]PAH was identical to the pattern showed by indomethacin, both in cortex and medulla. In cortex PGE$_2$ induced a decrease in PAH uptake from 3874 ± 149 to 889 ± 21 d min^{-1} mg^{-1} dry weight at the maximal concentration of PGE$_2$ tested (10^{-4} M). IC50 was $1{\cdot}3 \times 10^{-5}$ M, which is similar to that produced by indomethacin. Increasing concentrations of PGE$_2$ in the bathing media had no effect on medulla uptake supporting that the PAH uptake observed in cortex is a measurement of organic anions transport.

To evaluate this a kinetic analysis was carried out with varying concentrations of [^{14}C]PAH (from 1×10^{-7} to $1{\cdot}99 \times 10^{-6}$ M) and a fixed concentration of indomethacin (2×10^{-5} M). From a Lineweaver

Burke plot an apparent Michaelis constant (K_m) of 2·4 μM^{-1} for PAH and a K_m of 6·7 μM^{-1} for indomethacin were obtained (Fig. 4). The kinetic analysis is suggestive of a competitive inhibitory effect of indomethacin on the [^{14}C]PAH uptake.

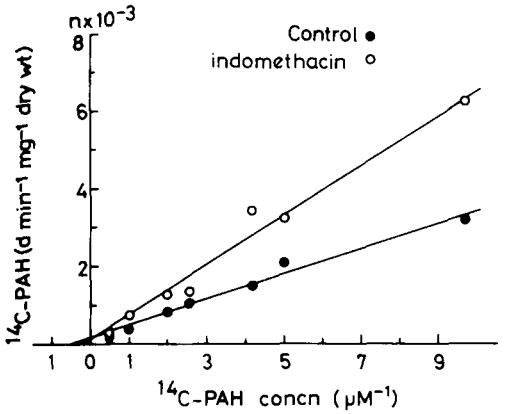

FIG. 4. The uptake of [^{14}C]PAH was measured in the presence (○—○) and in the absence (●—●) of indomethacin (2×10^{-5} M). [^{14}C]PAH concentrations ranged from 1×10^{-7} to $1{\cdot}99 \times 10^{-6}$ M. From a Lineweaver Burke plot a K_m for PAH of 2·4 μM^{-1} and a K_m of 6·7 μM^{-1} for indomethacin were obtained. Kinetic analysis is suggestive of a competitive inhibition of [^{14}C]PAH uptake by indomethacin.

DISCUSSION

The substances recognized as being transported by the acid secretory mechanism have not been endogenous (PAH, probenecid, penicillin and radiopaque media), however Bito et al (1976a) have suggested that the system also transports prostaglandins. Indomethacin is an effective inhibitor of prostaglandin synthetase in kidney and other tissues (Ferreira et al 1971; Berl et al 1977; Roman et al 1978; Haylor & Lote 1980; Hassid & Dunn 1980; Usberti et al 1980). As it had been proposed that indomethacin is secreted by the organic acid secretory mechanism, the possible interaction of prostaglandins, indomethacin and PAH on the secretory transport was studied.

Although PGE_2 was tested at a 1000-fold lower concentration that PAH (10^{-9} M and 10^{-6} M, respectively), the IC50 of indomethacin was only four times higher for PGE_2 than for PAH, suggesting that the affinity of the carrier for PGE_2 is higher than its affinity for PAH, as might be expected for a carrier involved both in the transport of endogenous as well as of exogenous compounds. We found the IC50 of indomethacin on [^{3}H]PGE_2 uptake (3·6 μM^{-1}) to be slightly lower than that by Bito & Salvador (1976b) for [^{3}H]$PGF_{2\alpha}$ (12 μM^{-1}).

The in vivo administration of indomethacin did not fully prevent the uptake [^{3}H]PGE_2 by the cortical slices as observed when additional indomethacin was present in the incubating media. It is possible that indomethacin did not completely inhibit prostaglandin synthesis and therefore some endogenously produced prostaglandins might be present and compete with both [^{3}H]PGE_2 and indomethacin for the carrier. If this were so, an increase in the [^{3}H]PGE_2 uptake should be observed at the higher concentrations of indomethacin in the incubating media, since cyclo-oxygenase would be inhibited and no competition would exist between endogenous and exogenous prostaglandins. Our data showed the opposite result, the lowest uptake was observed at the highest concentration of indomethacin, suggesting that the main inhibitory effect was due to a blocking action of indomethacin on the transporting system. Gafni et al (1978) reported that, in rabbits, the inhibition of cyclo-oxygenase by indomethacin lasts 4 to 6 h, the inhibition being complete even with doses lower than those we used. Furthermore, slices were tested within 1–2 h of the last dose of indomethacin, and therefore it was expected that the drug would keep its full effect at the time of the experiment.

Total PGE_2 uptake can be the result of several events such as carrier-mediated transport, receptor-specific binding, the presence of variable amounts of endogenously-produced prostaglandins and the effect of some metabolites on the transport, as has been suggested by Rennick (1977). Therefore if a study of the characteristics of the transport of indomethacin by the anion secretory pathway is being made, it is more accurate to use an indicator neither produced nor metabolized in the renal tissue. PAH is such an indicator and the effect of indomethacin on the accumulation of PAH by cortical slices is an adequate model. A clear inhibitory effect of indomethacin on PAH uptake was observed, in agreement with the results of [^{3}H]PGE_2 uptake, indicating that the effect is mainly due to inhibition of transport and not to some other action of indomethacin on the kidney (Dunn & Hood 1977).

Previous workers (Skeith et al 1968; Yesair et al 1970; Bito & Salvador 1976a, b) have not reported on the mechanism of inhibiton by indomethacin of PAH transport and have assumed that it is mediated by competition for the carrier. We show that the inhibitory effect of indomethacin on the PAH uptake is competitive, as has been described for probenecid or 2,4-dinitrophenol (Huang & Lin 1965; Berndt & Grote 1968; Berner & Kinne 1976).

A factor in the renal secretion of organic acids is the presence of an anion binding protein, ligandin (Kirsch et al 1975) within the proximal renal cells. We did not evaluate the possible participation of this protein on the transport.

The effect of prostacyclin on PAH uptake was not attempted because of the short half-life of this prostaglandin.

The role of prostaglandins on renal blood flow has been assessed by testing the effect of inhibiting their synthesis, mainly with indomethacin, and to measure the potential changes in blood flow by the extraction rate of PAH (Feigen et al 1976; Kramer et al 1980; Haylor & Lote 1980). The present study shows indomethacin and PAH share the same secretory pathway, therefore measurement of PAH extraction in the presence of indomethacin may give a misleading result. Also, prostaglandins themselves are transported through the same mechanism, making any assessment of their role on renal blood flow extremely difficult.

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