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Differential effects of sulindac on renal hemodynamics and function in the rat

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Abstract

Renal hemodynamics were studied using an electromagnetic perivascular flow sensor in anesthetized rats injected i.v. with vehicle, 5 or 10 mg/kg body weight (b.w.) sulindac. No hemodynamic changes occurred with vehicle ($n = 6$), but mean arterial pressure was significantly decreased (by 15 mmHg) with sulindac ($n = 12$). In the 5 mg/kg b.w. sulindac group ($n = 7$), renal blood flow progressively and significantly increased from 7.88 ± 0.36 to 8.98 ± 0.58 ml/min, except during concomitant intrarenal infusion of 3 mg/kg b.w. per h proadifen ($n = 7$). The pressure limits for efficient and no renal blood flow autoregulation remained unchanged (approx. 100 and 80 mmHg, respectively). In the 10 mg/kg b.w. sulindac group ($n = 5$), renal blood flow did not change but autoregulatory pressure limits were lowered by 10 mmHg 2 h after treatment ($P < 0.025$). Also, Na^+ retention was marked. Prostanoid excretion in urine was significantly reduced with either dose but basal plasma renin activity was not (about 8 ng/ml per h; $n = 15$). When plasma renin activity was enhanced after a reduction in renal perfusion pressure ($n = 21$), it was decreased from 11.5 ± 1.2 to 7.4 ± 0.2 ng/ml per h only by 10 mg/kg b.w. sulindac ($P < 0.05$; $n = 6$). In conclusion, differential effects of sulindac on renal hemodynamics, Na^+ excretion and plasma renin activity were demonstrated. Renal hemodynamic changes could be related in part to the cytochrome *P*-450 arachidonic acid pathway. © 1997 Elsevier Science B.V.

Keywords: Non-steroidal anti-inflammatory drug; Kidney; Circulation; Prostanoid; Plasma renin activity; Cytochrome *P*-450 activity; Na^+ excretion

1. Introduction

In general, the effects of cyclooxygenase inhibition on renal hemodynamics have been studied using indomethacin as the test drug. No marked changes in renal hemodynamics were previously observed in rats treated acutely with indomethacin, except in female rats during pregnancy (Baylis, 1987; Chevalier et al., 1987; Finn and Arendshorst, 1976). Recently, it has been shown that acute treatment with indomethacin could induce marked renal vasodilation and enhance the efficiency of renal blood flow autoregulation in anesthetized euvolemic male rats (Kramp et al., 1995). These hemodynamic changes in the kidney were dose- and time-dependent. They mainly occurred at a dose of 3 mg/kg body weight (b.w.) indomethacin injected i.v. which is lower than the dose generally used in studies on renal hemodynamics in the rat (Chevalier et al., 1987; Finn and Arendshorst, 1976). Interestingly, these findings occurred in the presence of an effective inhibition of cyclooxygenase activity and a decrease in plasma renin activity. At present, the factor(s) inducing these hemodynamic changes during cyclooxygenase inhibition remain(s) unknown.

Our observations on renal hemodynamic effects of indomethacin support the view of Abramson and Weissmann (1989) that the original hypothesis of Vane (1971), which restricted properties of anti-inflammatory drugs to cyclooxygenase inhibition, 'cannot be generalized further to all products of the arachidonic acid cascade and to all NSAIDs at all dosages'. Our aim was thus to further investigate renal hemodynamics using another non-steroidal anti-inflammatory drug (NSAID) for comparative pur-

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poses. To address this issue, sulindac, an NSAID of the same chemical class as indomethacin, was chosen because sulindac presents some specific properties and its renal hemodynamic effects have not yet been characterized. Sulindac is a prodrug, sulindac sulfoxide, which has to be converted to the active metabolite, sulindac sulfide, to effectively inhibit cyclooxygenase activity (Duggan et al., 1978). The inhibition of cyclooxygenase by sulindac is reversible (Smith et al., 1991). Interestingly, sulindac sulfide does not appear in the urine because sulindac sulfide is reconverted rapidly to sulindac sulfoxide in the kidney (Miller et al., 1984). Therefore, it has been postulated that sulindac may be less effective than other NSAIDs to inhibit cyclooxygenase activity in the kidney (Ciabattoni et al., 1984). In the human and in the rat, the plasma half-life of sulindac is long compared to that of indomethacin because of the metabolic specificities and the entero-hepatic recirculation of sulindac sulfoxide (Duggan et al., 1978). Contrasting with the hyporeninemic effects of other NSAIDs, sulindac does not reduce plasma renin activity in the human, at least when the drug is administered in the therapeutic dose range, nor in the rat, except when rats were put on a low sodium diet and given a high oral dose of sulindac (32 mg/kg b.w.) (Cinotti et al., 1984; Izumi et al., 1985; Romero et al., 1976).

The purpose of our study was to investigate differential effects of sulindac on renal hemodynamics and function as well as to evaluate the effectiveness of the drug on plasma renin activity and on the synthesis of prostanoids. Moreover, the hypothesis that NSAIDs might interact with some vasoactive product(s) of the cytochrome *P*-450 arachidonic acid pathway was also considered (McGiff, 1991). Two doses of sulindac were selected for study in anesthetized male rats. A dose of 5 mg/kg b.w. sulindac was chosen because it presents an anti-inflammatory ED_{50} in the rat (Duggan et al., 1978). A dose of 10 mg/kg b.w. sulindac was tested because it is comparable, on a dose basis, to the higher doses of indomethacin and meclofenamate previously administered to investigate renal hemodynamics in rats (Chevalier et al., 1987; Finn and Arendshorst, 1976; Kramp et al., 1995).

2. Materials and methods

2.1. Animal preparation

Prior to experimentation, male Wistar rats weighing $\pm$300 g and maintained on a rat chow diet (25 g daily of Muracon G. Ster. containing 1.8 g Na^+ and 9.6 g of K^+ per kg, Trouw, Gent, Belgium) were deprived of food overnight but had free access to tap water. They were anesthetized with Inactin (Byk-Gulden), 10 mg/100 g b.w., injected i.p. The animals were placed on a heated table to maintain rectal temperature between 37° and 38°C. The left femoral artery was first catheterized to determine hematocrit, plasma electrolytes and to measure mean arterial pressure. Mean arterial pressure was measured using a Statham P23 ID pressure transducer connected to a pressure monitor (Mennen Medical) and a recorder. Then, the right femoral artery, for subsequent blood sampling, and vein were rapidly catheterized. To avoid fluid shifts during further surgery, a 0.85% NaCl solution containing 2.5% albumin was immediately infused into the right femoral vein at a rate of 150 μl/min for 5 min and of 83 μl/min during the next 30 min. This infusion was maintained throughout experimentation at a rate of 8 μl/min. Following tracheostomy, the right jugular vein was catheterized for subsequent infusions. The left kidney was exposed through a midline and subcostal abdominal incision as previously described (Kramp and Lenoir, 1975). The segment of the aorta located between the two renal arteries as well as the left renal artery were then carefully dissected from surrounding tissues avoiding as much as possible interference with nerve bundles. When appropriate, an adjustable constriction clamp was placed around the aorta between the left and the right renal arteries to reduce renal perfusion pressure. Finally, each ureter was cannulated for urine collection. In some rats, a tapered and curved PE 10 catheter was introduced into the left iliac artery. The catheter was advanced through the abdominal aorta and its tip was positioned in the left renal artery as described by Chatziantoniou and Arendshorst (1992). Lissamine green was injected into the catheter to verify the uniform distribution of the dye on the entire surface of the kidney. The experiment was discontinued when the dye was only distributed to a portion of the kidney. At the end of the experiment, lissamine green was again injected in the renal artery to verify distribution of the dye to the entire kidney. A 0.85% NaCl solution was continuously infused into the renal arterial catheter of these rats at a rate of 5 μl/min. After completion of surgery and following suitable priming, a 0.85% NaCl solution containing 3% inulin (Laevosan, Innsbruck, Austria) was infused at a rate of 48 μl/min to measure glomerular filtration rate, except if otherwise stated. At the end of the experiment, the kidneys were decapsulated, blotted dry and weighed.

A small-diameter non-cannulating and factory-precalibrated electromagnetic flow transducer (0.6 mm internal diameter) connected to a square-wave electromagnetic flowmeter (MDL 1401 compact, Skalar Medical, Delft, Netherlands) and a recorder, was vertically fitted around the left renal artery for continuous measurement of renal blood flow. Calibration and use of these flow sensors as well as the final tests to check the accuracy of renal blood flow measurements at the end of the experiment were previously described in detail (Kramp et al., 1995). No marked changes in renal blood flow measurements before or after these final tests were found (change = 0.3 $\pm$ 1.5%). Note that renal blood flow measurements were not affected by renal artery catheterisation of the left kidney. Renal blood flow autoregulatory efficiency was investigated by

stepwise aortic constrictions inducing 5-mmHg decrements in pressure from the spontaneous mean arterial pressure down to 60 mmHg and by measurement of renal blood flow during 30-s periods. Mean arterial pressure was arbitrarily considered to represent renal perfusion pressure. No measurements were undertaken during increased mean arterial pressure.

In other rats surgically prepared as described above, the right carotid artery and the left femoral vein were catheterized to rapidly collect 1–1.5 ml of arterial blood for determination of arterial plasma renin activity and to replace the blood withdrawn by an equivalent volume of blood from a donor rat. A small catheter was introduced into the suprarenal vein to collect renal venous blood in some of these rats to analyze renal renin activity. After completion of surgery, a 0.85% NaCl solution without inulin was infused in each rat at a rate of 48 μl/min until the end of the experiment. Littermates, selected as donor rats, were also anesthetized with 10 mg/100 g b.w. Inactin, injected i.p. Thereafter, the carotid artery was catheterized and approximately 5 ml of blood was rapidly collected in tubes containing sodium ethylene diamine tetraacetic acid (EDTA). The temperature of the blood sample was maintained at 37°C until transfusion. After blood withdrawal, the donor rats were immediately killed with a lethal dose of Nembutal.

2.2. Experimental protocols

After 60- to 90-min equilibration, baseline measurements of hemodynamics and renal function were carried out during 3 control periods of 20 min each. Control autoregulatory maneuvers were undertaken during the second control period. Then, chosen at random, vehicle (0.5 ml of isotonic saline), 5 or 10 mg/kg b.w. of water-soluble powder of sulindac (Merck, Sharp & Dohme) dissolved in 0.5 ml of saline was injected i.v. at a rate of 50 μl/min for 10 min followed by a 20-min period of equilibration. Six experimental periods of 20-min duration each then followed. Autoregulatory maneuvers were again carried out during the second and fifth experimental periods. In each rat, blood was periodically sampled from the femoral artery, and urine was collected separately from each kidney at the end of each period for the determination of inulin clearance and of electrolyte and prostanoid excretion rates. A final blood sample was withdrawn for analysis of plasma electrolytes after the accuracy of renal blood flow measurements was tested. An experimental protocol of similar duration was used for the rats with renal arterial catheterisation of the left kidney but no autoregulatory maneuvers were undertaken and the duration of each period was reduced to 15 min. Following baseline measurements, proadifen (Sigma) dissolved in isotonic saline was infused intrarenally at a rate of 3 mg/kg b.w. per h to inhibit the cytochrome *P*-450 arachidonic acid pathway (Harder et al., 1995). This dose presumably corresponds to an intrarenal vascular concentration of about 10 μM proadifen (Kauser et al., 1991). In some of these rats, 5 mg/kg b.w. sulindac was injected i.v. as described above at the start of the intrarenal infusion of proadifen.

The effects of sulindac on plasma renin activity were investigated in other rats. Again, three control periods of 20-min duration each were carried out after 60–90 min post-surgical equilibration. At the start of the second control period, blood was sampled from the carotid artery or from the suprarenal vein to determine baseline systemic or renal venous plasma renin activity. The collected blood volume was immediately replaced with an equivalent volume of donor blood. Much care was taken with the blood transfusion to detect possible adverse reactions. At the end of the control periods, vehicle (0.5 ml of saline) or sulindac at a dose of 5 or 10 mg/kg b.w. was injected i.v. over 10 min followed by 20-min equilibration as described above. Then, six experimental periods of 20-min duration each were undertaken in one group of rats. At the end of the experiment, blood was again withdrawn from the carotid artery or the suprarenal vein for determination of plasma renin activity. In another group of rats, renal perfusion pressure was reduced to 80 mmHg by constriction of the abdominal aorta after the third experimental period. Renal perfusion pressure was maintained at 80 mmHg during 20 min. Thereafter, blood was withdrawn from the carotid artery and immediately replaced with donor blood. Arterial blood was again withdrawn at the end of the experiment. In each rat, urine was collected during control and experimental periods to measure urine flow and to determine the excretion rate of sodium and of prostanoids. Mean arterial pressure was measured continuously and the hematocrit was determined periodically.

2.3. Experimental groups

The following experimental groups were used to study the effects of sulindac:

(A) On renal hemodynamics and function: (1) 5 rats were treated i.v. with vehicle (0.5 ml of solvent); (2) 7 rats were treated i.v. with sulindac at a dose of 5 mg/kg b.w.; (3) 5 rats were treated i.v. with sulindac at a dose of 10 mg/kg b.w.

(B) On plasma renin activity: (4) 15 rats were treated i.v. with vehicle (0.5 ml of solvent) in the absence ($n = 8$) or in the presence of a transient reduction of renal perfusion pressure ($n = 7$); (5) 15 rats were treated i.v. with sulindac at a dose of 5 mg/kg b.w. in the absence ($n = 7$) or in the presence of a transient reduction of renal perfusion pressure ($n = 8$); (6) 14 rats were treated i.v. with sulindac at a dose of 10 mg/kg b.w. in the absence ($n = 8$) or in the presence of a transient reduction of renal perfusion pressure ($n = 6$).

(C) On the intrarenal treatment with proadifen: (7) 5 rats were treated with isotonic saline infused in the left renal artery at a rate of 5 μl/min; (8) 7 rats were treated

with proadifen infused in the left renal artery at a rate of 3 mg/kg b.w. per h; (9) 6 rats were treated simultaneously with proadifen infused in the left renal artery at a rate of 3 mg/kg b.w. per h and with sulindac injected i.v. at a dose of 5 mg/kg b.w.

2.4. Analytical methods

Urine volume from each kidney was estimated by gravimetry assuming a specific gravity of 1.0 for water. Inulin in plasma and urine samples was determined using the anthrone method (Davidson and Sackner, 1963). Plasma and urine sodium and potassium concentrations were measured by flame photometry (Klina, Beckman, or IL-943, Instrumentation Laboratory). Plasma and urine osmolalities were determined with a micro-osmometer (Advanced micro-osmometer). Prostaglandins E_2, $F_{2\alpha}$ and 6-keto-$F_{1\alpha}$ as well as thromboxane B_2 in urine samples were measured by radioimmunoassay (Granström and Kindahl, 1978). Handling of the urine samples and of standards was previously described in detail (Kramp et al., 1995). Cross-reactivity of the anti-sera was determined at 50% of total binding and was $\leq 1\%$, except between prostaglandins $F_{2\alpha}$ and 6-keto-$F_{1\alpha}$ (6.9%). Minimal detection limits of prostaglandins E_2, $F_{2\alpha}$, 6-keto-$F_{1\alpha}$ and thromboxane B_2 were 15, 15, 2 and 2 pg, respectively. Intra- and interassay variation coefficients were calculated from the pairs of samples and were $\leq 1\%$. The dose interpolation was calculated using a four-parameter model (Dudley et al., 1985). No significant differences in prostanoid excretion were observed between kidneys, except during the periods of autoregulatory maneuvers or reduced renal perfusion pressure. These periods were not included in the calculations. Blood from the carotid artery or from the suprarenal vein was collected in ice-cooled tubes containing EDTA for determination of plasma renin activity on a 0.5-ml sample. Plasma renin activity was determined by radioimmunoassay using ^{125}I-angiotensin I (Medic Angiotensin I test) (Fyhrquist et al., 1976). Intra- and interassay variability was less than 10 and 20%, respectively.

2.5. Calculations and statistics

Glomerular filtration rate, filtration fraction and renal excretory function were calculated as previously described (Pitts, 1968). Mean results for glomerular filtration rate, filtration fraction, diuresis, sodium and potassium excretion rates, urine osmolality and fractional excretion of Na^+ and of K^+ represent the average of measurements from two control and four experimental periods which correspond respectively to the pre- and post-autoregulatory periods, as indicated in the experimental protocol. Data obtained during the autoregulatory periods were not included in the average since autoregulatory maneuvers transiently reduced glomerular filtration rate and the excretory function from the left kidney, henceforth referred to as the experimental kidney. Except if otherwise stated, results are presented as the sum of the left and right kidney values. Renal hemodynamic data were obtained from the experimental kidney. Renal plasma flow was estimated as the product of renal blood flow and (1 − hematocrit). Renal vascular resistance was calculated as the ratio between mean arterial pressure and renal blood flow, where mean arterial pressure represents the mean arterial pressure in the femoral artery. Renal perfusion pressure was arbitrarily equated to the femoral mean arterial pressure. Mean values for renal blood flow, mean arterial pressure and renal vascular resistance were obtained by averaging hand-made measurements carried out visually, on the recordings, every 5 min except during autoregulatory maneuvers. The latter established the relationship between renal blood flow and mean arterial pressure which was assessed in each experiment by linear regression using the least-squares method (Persson et al., 1988). The size of the correlation coefficient, the slope and the intercept of the best-fit regression lines were taken as criteria to attribute a data point to one of the following components of the relationship between renal blood flow and mean arterial pressure: (1) the autoregulatory plateau, which corresponds to the pressure range with unchanged or less than 3% change in renal blood flow; (2) a subautoregulatory zone, which corresponds to the pressure range where some autoregulatory efficiency persisted; and (3) absence of autoregulation, which corresponds to the pressure range where renal blood flow changes were fully pressure-dependent. The perfusion pressure corresponding to the lower limit of the autoregulatory plateau was defined as the pressure limit of efficient renal blood flow autoregulation. The perfusion pressure corresponding to the disappearance of renal blood flow autoregulation was defined as the pressure limit of no renal blood flow autoregulation. Autoregulatory curves were normalized and are presented as percents of control renal blood flow measured at a perfusion pressure of 105 mmHg.

One-way analysis of variance was applied for multiple intergroup comparisons and one-way analysis of variance for repeated measurements was applied for multiple intragroup comparisons. Significant differences were identified with a post-hoc *t*-test. Two-way analysis of variance for repeated measurements on one factor was used to compare plasma renin activity between groups (Wallenstein et al., 1980). Significant differences were identified with the Newman-Keuls test for multiple comparisons. Differences between two groups were tested with the unpaired *t*-test. The paired *t*-test was used for a single comparison within a group. The relationship between hemodynamic variables (mean arterial pressure and renal blood flow) and time was analysed using linear regressions with the best-fit line determined by the method of least squares. The regression equations were written as $Y = b_0 + b_1 X + b_2 X^2 + \ldots b_n X^n$. They were completed with the correlation coefficient (r) and significance. All results are presented

as mean values ± S.E.M. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. General observations

Body weight and left and right kidney weights did not differ markedly between experimental groups. They averaged 292 ± 1, 1.149 ± 0.017 and 1.157 ± 0.021 g in 81 rats, respectively. Plasma sodium, potassium and osmolality were in the normal range for rats (data not shown). They did not differ before or after each treatment. No time-related changes in hematocrit occurred in the 25 rats treated with vehicle. Initial and final hematocrit averaged, respectively, 45.4 ± 0.5 and 44.8 ± 0.6% (NS). In contrast, the hematocrit was significantly reduced from 45.2 ± 0.6 to 42.1 ± 0.6% ($P < 0.001$) in 22 rats treated with 5 mg/kg b.w. sulindac and from 43.7 ± 0.5 to 40.2 ± 0.6% ($P < 0.001$) in 19 rats treated with 10 mg/kg b.w. sulindac. The hematocrit remained stable during the intrarenal infusion of proadifen and averaged 44.1 ± 0.4 before and 43.7 ± 0.5% at the end of the infusion (NS; $n = 7$). However, the hematocrit decreased significantly from 42.3 ± 1.0 to 40.1 ± 1.1% when the animals were treated simultaneously with proadifen infused intrarenally and with sulindac injected i.v. at a dose of 5 mg/kg b.w. ($P = 0.008$; $n = 6$).

3.2. Hemodynamics

Baseline mean arterial pressure, renal blood flow and renal vascular resistance averaged, respectively, 117 ± 2 mmHg, 7.79 ± 0.53 ml/min and 15.4 ± 1.3 mmHg/ml per min in 5 rats treated with vehicle, 120 ± 3 mmHg, 7.92 ± 0.40 ml/min and 15.3 ± 0.8 mmHg/ml per min in 7 rats treated with 5 mg/kg b.w. sulindac and 123 ± 3 mmHg, 8.40 ± 0.60 ml/min and 14.9 ± 1.2 mmHg/ml per min in 5 rats treated with 10 mg/kg b.w. sulindac. There were no statistically significant differences between the three groups. Fig. 1 illustrates the temporal variations of mean arterial pressure and renal blood flow starting just before the injection of vehicle or sulindac until the end of the 2-h experimental periods. As illustrated, there were no significant changes in mean arterial pressure or renal blood flow related to the injection of vehicle, or to time. These averaged, respectively, 119 ± 2 mmHg and 7.65 ± 0.58 ml/min prior to and 116 ± 2 mmHg and 7.54 ± 0.45 ml/min at the end of the experimental periods. Conversely, significant time-related changes in mean arterial pressure and renal blood flow occurred in the rats treated with 5 mg/kg b.w. sulindac ($P < 0.001$). Mean arterial pressure decreased from 120 ± 3 to 107 ± 4 mmHg ($P < 0.05$) while renal blood flow increased from 7.88 ± 0.36 to 8.98 ± 0.58 ml/min (change = 14%; $P < 0.05$) 90 min after the start of the treatment. Thereafter, mean arterial pressure and renal blood flow remained stable and averaged, respectively, 108 ± 4 mmHg ($P < 0.05$ from pretreatment) and 8.83 ± 0.62 ml/min (change = 12%; $P < 0.05$ from pretreatment) at the end of the experiment. Significant time-related changes occurred only in mean arterial pressure in the rats treated with 10 mg/kg b.w. sulindac ($P < 0.001$). Mean arterial pressure decreased from 122 ± 3 to 110 ± 3 mmHg ($P < 0.05$) 90 min after the start of the treatment and thereafter remained stable until the end of the experiment (111 ± 3 mmHg; $P < 0.05$ from pretreatment). In contrast, renal blood flow did not vary markedly with time, averaging 8.26 ± 0.67 ml/min prior to the injection of sulindac and 7.99 ± 0.31 ml/min at the end of the experiment. Renal vascular resistance at the end of the experiment averaged 15.6 ± 1 (NS from baseline), 12.1 ± 0.6 ($P = 0.018$) and 14 ± 0.8 mmHg/ml per min (NS) in the rats injected with vehicle, 5 or 10 mg/kg b.w. sulindac, respectively. Fig. 2 summarizes the differential effects of sulindac exerted on renal vascular

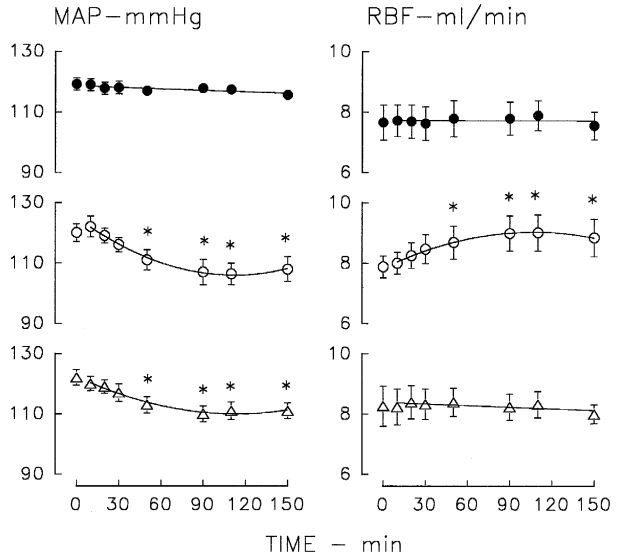

Fig. 1. Temporal variations of mean arterial pressure (MAP, in mmHg) and renal blood flow (RBF, in ml/min) in rats treated with vehicle (●), 5 (○) or 10 (△) mg/kg b.w. sulindac. Mean values ± S.E.M. are given for just before and 10, 20, 30, 50, 90, 110 and 150 min after drug treatment. Differences in temporal variations of mean arterial pressure or renal blood flow were evaluated by analysis of variance for repeated measurements followed by Newman-Keuls test comparing pre- and post-injection values. Statistical significance: $P < 0.001$, analysis of variance; * $P < 0.05$, Newman-Keuls test. Best-fit linear regression between mean arterial pressure or renal blood flow and time was calculated for post-treatment measurements. Relationships in rats treated with vehicle corresponded, respectively, to the equations: $Y = 118.6 - 0.016X$ ($r = 0.808$; $P = 0.03$) and $Y = 7.72 - 9.9 \times 10^{-5} X$ ($r = 0.046$; NS). Relationships in rats treated with 5 mg/kg b.w. sulindac corresponded, respectively, to the equations: $Y = 125.0 - 0.342X + 1.53 \times 10^{-3} X^2$ ($r = 0.998$; $P < 0.001$) and $Y = 7.83 + 0.022X - 1.05 \times 10^{-4} X^2$ ($r = 0.997$; $P < 0.001$). Relationships in rats treated with 10 mg/kg b.w. sulindac corresponded, respectively, to the equations: $Y = 122.4 - 0.221X + 9.77 \times 10^{-4} X^2$ ($r = 0.987$; $P < 0.001$) and $Y = -0.0018X + 8.385$ ($r = 0.829$; NS). Injection time of vehicle or sulindac: from 0 to 10 min.

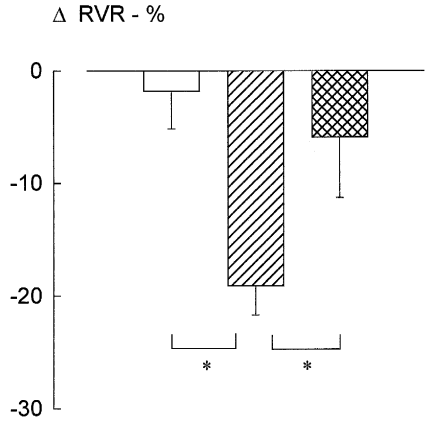

Fig. 2. Percent changes in renal vascular resistance (ΔRVR in %) 2 h after injection of vehicle (open bar), 5 (hatched bar) or 10 (cross-hatched bar) mg/kg b.w. sulindac. Mean values ± S.E.M. are illustrated. Statistical significance: $P = 0.01$, analysis of variance; * $P < 0.05$, Newman-Keuls test.

Table 1
Pressure limits of efficient, and absent, renal blood flow autoregulation before (A_1) and 1 (A_2) and 2 h (A_3) after vehicle or sulindac infusion

Autoregulatory period	P_A (mmHg)	P_0 (mmHg)
Vehicle (n = 5)		
A_1	100 ± 2	81 ± 3
A_2	97 ± 2	83 ± 4
A_3	98 ± 1	80 ± 2
5 mg / kg b.w. sulindac (n = 7)		
A_1	102 ± 2	81 ± 3
A_2	102 ± 1	82 ± 2
A_3	100 ± 3	83 ± 2
10 mg / kg b.w. sulindac (n = 5)		
A_1	98 ± 4	84 ± 2
A_2	95 ± 3	81 ± 3
A_3	89 ± 3 [a]	73 ± 2 [a]

Mean results ± S.E.M. are presented. Statistical significance versus A_1 within a group (analysis of variance followed by Bonferroni *t*-test): [a] $P < 0.05$. No statistical difference in control (A_1) values for P_A or P_0 between the three experimental groups (NS, analysis of variance). P_A = pressure at lower limit of efficient renal blood flow autoregulation; P_0 = pressure limit at no renal blood flow autoregulation. n = number of rats.

resistance expressed as percent changes from pretreatment values. The change in renal vascular resistance averaged respectively -2 ± 3, -19 ± 3 and -6 ± 5% in the three groups ($P = 0.01$). Only the change in renal vascular resistance induced by the low dose of sulindac differed significantly from the change in the two other groups ($P < 0.05$).

Fig. 3 illustrates the renal blood flow-mean arterial pressure relationship 30 min before and 1 and 2 h after the i.v. injection of vehicle, 5 or 10 mg/kg b.w. sulindac. The autoregulatory curves before and after treatment with vehicle were similar (Fig. 3A). In contrast, the autoregulatory curves after the injection of 5 mg/kg b.w. sulindac were shifted upward from the control when the renal perfusion pressure was ≥ 85 mmHg (Fig. 3B). The three autoregulatory curves, however, did not differ from each other when renal perfusion pressure was ≤ 85 mmHg. The autoregulatory curves before and 1 h after the injection of 10 mg/kg b.w. sulindac were similar (Fig. 3C). In contrast, the autoregulatory plateau was prolonged 2 h after drug treatment. The renal blood flow-mean arterial pressure relationship was then shifted to the left when renal perfusion pressure was < 90 mmHg. As shown in Table 1, the lower pressure limit of efficient renal blood flow autoregulation of the control curves occurred approxi-

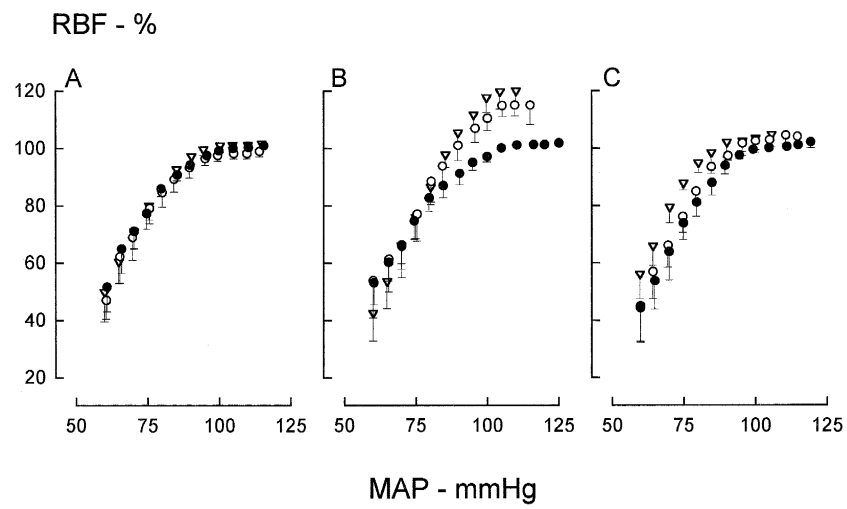

Fig. 3. Renal blood flow (RBF in %)-mean arterial pressure (MAP in mmHg) relationships during stepwise decreases in renal perfusion pressure in rats treated with vehicle (panel A), 5 (panel B) or 10 mg/kg b.w. (panel C) sulindac. Autoregulatory curves were obtained before (●) and 1 (○) and 2 h (▽) after vehicle or drug injection. Pre-autoregulatory renal blood flow values averaged (in ml/min), respectively, 7.75 ± 0.53, 7.63 ± 0.54 and 7.46 ± 0.46 in the vehicle group, 7.89 ± 0.39, 8.73 ± 0.54 and 9.08 ± 0.60 in the 5 mg/kg b.w. sulindac group ($n = 7$) and 8.40 ± 0.60, 8.40 ± 0.45 and 8.38 ± 0.33 in the 10 mg/kg b.w. sulindac group ($n = 5$). Mean values ± S.E.M. are illustrated. Some S.E.M. values are too small to be shown.

mately at 100 mmHg in the three groups while renal blood flow became fully pressure-dependent at a renal perfusion pressure of 80–85 mmHg. There were no further marked changes of these pressure limits with time in the vehicle and 5 mg/kg b.w. sulindac groups. However, the pressure limits were significantly lowered by 10 mmHg 2 h after injection of the high dose of sulindac ($P < 0.025$).

3.3. Glomerular filtration rate and salt and water excretion

Glomerular filtration rate and filtration fraction of the experimental kidney as well as diuresis, sodium and potassium excretion rates, urine osmolality and fractional excretion of sodium and potassium of the two kidneys before and after vehicle or sulindac treatment are shown in Table 2. The control values did not differ significantly between the three experimental groups. All the variables, except Na^+, remained stable after vehicle treatment. The excretion rate and fractional excretion of Na^+ were slightly enhanced. The enhancement did not reach statistical significance and was presumably due to Na^+ retention induced by the autoregulatory maneuvers as previously discussed (Kramp et al., 1995). Glomerular filtration rate and filtration fraction remained relatively stable throughout the period of experimentation in the two groups treated with sulindac. Diuresis was significantly reduced (by 43%) while the excretion rate and fractional excretion of Na^+ did not change in the rats injected with 5 mg/kg b.w. sulindac. Diuresis as well as excretion rate and fractional excretion of Na^+ were significantly diminished, respectively, by 60, 55 and 53% in the rats treated with the high dose of sulindac. In these rats, the excretion rate of Na^+ decreased rapidly from 6.04 ± 0.93 (last control period) to 3.04 ± 0.34 μEq/min (first experimental period; $P < 0.05$) and was still reduced at the end of the experiment (4.44 ± 0.57 μEq/min; $P < 0.05$ from the last control period). The differential effects of sulindac on the rate of sodium excretion in the urine were significant when expressed as absolute changes in sodium excretion rate from baseline ($P < 0.001$). These averaged 2.648 ± 1.280, -0.403 ± 0.547 ($P < 0.05$ from vehicle) and -3.789 ± 0.535 μEq/min ($P < 0.005$ from vehicle) in rats injected with vehicle, 5 or 10 mg/kg b.w. sulindac, respectively. In contrast, the excretion rate and fractional excretion of K^+ did not change irrespective of the dose of sulindac. Finally, urine osmolality was significantly enhanced, by 76%, only in the rats treated with the high dose of sulindac.

3.4. Plasma renin activity

Fig. 4 illustrates the systemic arterial or renal venous plasma renin activity determined in each rat before and 150 min after the injection of vehicle ($n = 8$), 5 ($n = 7$) or 10 ($n = 8$) mg/kg b.w. sulindac, respectively. Plasma renin activity in systemic arterial or renal venous blood averaged respectively 9.1 ± 2.2 ($n = 6$) and 8.2 ± 2.2 ($n = 2$) before and 9.0 ± 1.4 and 9.7 ± 3.5 ng/ml per h after treatment with vehicle (Fig. 4A). Rather than being lowered by sulindac at the end of each experiment ($n = 15$), it was slightly increased in most experiments ($n = 11$). Average plasma renin activity in systemic arterial or renal

Table 2
Glomerular filtration rate, filtration fraction, salt and water excretion, urine osmolality, fractional excretion of Na^+ and K^+ before and after vehicle or sulindac infusion

		Vehicle ($n = 5$)	5 mg/kg b.w. sulindac ($n = 7$)	10 mg/kg b.w. sulindac ($n = 5$)
GFR_{EK} (ml/min)	C	1.245 ± 0.039	1.092 ± 0.074	1.248 ± 0.057
	E	1.258 ± 0.054	1.221 ± 0.096	1.351 ± 0.082
FF_{EK}	C	0.30 ± 0.02	0.26 ± 0.01	0.27 ± 0.02
	E	0.31 ± 0.02	0.25 ± 0.01	0.29 ± 0.01
UV (μl/min)	C	32 ± 8	35 ± 6	35 ± 4
	E	32 ± 8	20 ± 3 [a]	14 ± 2 [a]
$U_{Na}V$ (μEq/min)	C	4.644 ± 1.300	4.817 ± 0.958	6.911 ± 0.635
	E	7.289 ± 2.051	4.414 ± 0.740	3.122 ± 0.172 [a]
U_KV (μEq/min)	C	1.929 ± 0.245	1.937 ± 0.153	2.285 ± 0.296
	E	2.360 ± 0.221	2.280 ± 0.154	2.057 ± 0.235
U_{osm} (mOsm/kg H_2O)	C	583 ± 156	754 ± 77	841 ± 69
	E	924 ± 99	1179 ± 105	1481 ± 155 [a]
FE_{Na} (%)	C	1.3 ± 0.4	1.5 ± 0.3	1.9 ± 0.3
	E	1.9 ± 0.5	1.3 ± 0.2	0.9 ± 0.1 [a]
FE_K (%)	C	21 ± 3	23 ± 3	24 ± 3
	E	25 ± 3	25 ± 3	23 ± 2

Mean results ± S.E.M. are presented. Statistical significance of differences between control (C) and experimental (E) results within a group by paired *t*-test ([a] $P < 0.01$). Control values did not differ between experimental groups (NS, analysis of variance). Values for GFR_{EK} and FF_{EK} relate to the glomerular filtration rate and the filtration fraction of the experimental kidney (EK). GFR of the contralateral kidney (data not shown) did not differ from GFR of the experimental kidney. UV, $U_{Na}V$, U_KV, U_{osm}, FE_{Na} and FE_K represent respectively urine flow, sodium and potassium excretion rates, urine osmolality and fractional excretion of sodium and potassium from the two kidneys. n = number of rats.

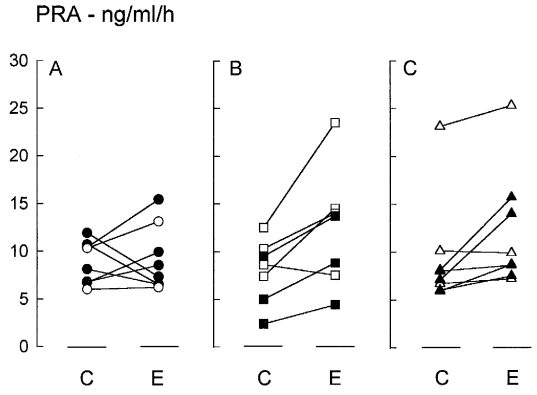

Fig. 4. Systemic arterial (closed symbols) and renal venous (open symbols) plasma renin activity (PRA in ng/ml/h) before (C) and after (E) infusion of vehicle (●, ○; panel A), 5 (■, □; panel B) or 10 (▲, △; panel C) mg/kg b.w. sulindac. Individual values are illustrated. Note that some values are superimposed in panel C.

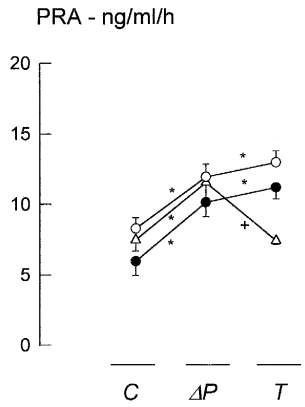

Fig. 5. Effect of reduced renal perfusion pressure on plasma renin activity (PRA, in ng/ml/h) in rats treated with vehicle (●; $n = 7$), 5 (○; $n = 8$) or 10 (△; $n = 6$) mg/kg b.w. sulindac. Mean values ± S.E.M. of baseline plasma renin activity (C), of PRA after RPP reduction (ΔP) and at the end of the experiment (T) are illustrated. Statistical significance was evaluated by two-way analysis of variance and Newman-Keuls test for multiple pairwise comparisons. Differences in mean values after allowing for differences in groups were significant ($P < 0.001$). Interaction between groups and treatment was also significant ($P = 0.001$). Equal variance test was NS. * $P < 0.05$ versus baseline, and † $P < 0.05$ between T and ΔP periods (Newman-Keuls test). Some S.E.M. are too small to be shown.

venous blood was, respectively, 5.6 ± 2.1 ($n = 3$) and 9.7 ± 1.1 ($n = 4$) before and 9.0 ± 2.7 and 14.9 ± 3.3 ng/ml per h after treatment with 5 mg/kg b.w. sulindac (Fig. 4B). Average plasma renin activity in systemic arterial or renal venous blood was, respectively, 6.8 ± 0.5 ($n = 4$) and 12.0 ± 3.8 ($n = 3$) before and 11.5 ± 2.0 and 12.8 ± 4.2 ng/ml per h after treatment with 10 mg/kg b.w. sulindac (Fig. 4C). In each rat, the time course of changes in mean arterial pressure, diuresis and natriuresis after the injection of vehicle or sulindac was similar to that described above (data not shown). Fig. 5 illustrates the effect of reducing renal perfusion pressure to 80 mmHg for 20 min on plasma renin activity in rats injected with vehicle ($n = 7$), 5 ($n = 8$) or 10 ($n = 6$) mg/kg b.w. sulindac. Baseline plasma renin activity averaged respectively 6.0 ± 1, 8.3 ± 0.8 and 7.4 ± 0.6 ng/ml per h in each group (NS). Following treatment with vehicle or with 5 or 10 mg/kg b.w. sulindac, plasma renin activity was significantly elevated by reducing renal perfusion pressure and averaged, respectively, 10.1 ± 1, 11.9 ± 0.9 and 11.5 ± 1.2 ng/ml per h ($P < 0.05$ from each baseline). At the end of the experiment, plasma renin activity remained enhanced in the rats treated with vehicle (11.2 ± 0.8 ng/ml per h) or with 5 mg/kg b.w. sulindac (13 ± 0.8 ng/ml per h) and differed significantly from baseline ($P < 0.05$). In contrast, plasma renin activity at the end was significantly diminished (to 7.4 ± 0.2 ng/ml per h) in the rats treated with the high dose of sulindac ($P < 0.05$, from preceding value). It was then again similar to its baseline value.

3.5. Cyclooxygenase inhibition

Table 3 presents the excretion rates of prostaglandins E_2, $F_{2\alpha}$, 6-keto-$F_{1\alpha}$ and thromboxane B_2 in urine, before and after the injection of vehicle ($n = 6$), 5 ($n = 12$) or 10 ($n = 11$) mg/kg b.w. sulindac, measured in rats submitted to autoregulatory maneuvers or used for determination of plasma renin activity with reduction of renal perfusion pressure. Note that the calculations did not include the experimental periods with mechanical changes in pressure. Excretion rates of prostaglandins E_2, $F_{2\alpha}$, 6-keto-$F_{1\alpha}$ and

Table 3
Effects of sulindac on excretion rates of prostanoids in urine

		Vehicle ($n = 6$)	% Δ	5 mg/kg b.w. sulindac ($n = 12$)	% Δ	10 mg/kg b.w. sulindac ($n = 11$)	% Δ
PGE_2	C	0.361 ± 0.114		0.447 ± 0.066		0.446 ± 0.123	
	E	0.309 ± 0.045	14	0.139 ± 0.019 [b]	69	0.062 ± 0.009 [a]	86
$PGF_{2\alpha}$	C	0.117 ± 0.020		0.259 ± 0.057		0.214 ± 0.052	
	E	0.114 ± 0.015	3	0.071 ± 0.013	73	0.042 ± 0.006 [a]	80
6-keto-$PGF_{1\alpha}$	C	0.317 ± 0.091		0.479 ± 0.072		0.317 ± 0.068	
	E	0.249 ± 0.053	21	0.129 ± 0.018 [b]	73	0.068 ± 0.012 [a]	79
TxB_2	C	0.072 ± 0.017		0.107 ± 0.023		0.083 ± 0.015	
	E	0.069 ± 0.018	4	0.074 ± 0.024	31	0.042 ± 0.009 [b]	49

Values are means ± S.E.M. and are expressed as ng/min/kidney. Statistical significance of differences between control (C) and experimental (E) results within a group (paired t-test): [a] $P < 0.01$; [b] $P < 0.001$. n = number of rats. % Δ, average percent decrease; PG, prostaglandin; TxB_2, thromboxane B_2.

thromboxane B_2 in urine did not vary markedly after vehicle treatment. In contrast, excretion rates of prostaglandins E_2, $F_{2\alpha}$ and 6-keto-$F_{1\alpha}$ were rapidly decreased (< 1 h) after the injection of sulindac. They stabilized 90 min after the injection of the drug and were not reversed until the end of the experiment. However, excretion rates of thromboxane B_2 in urine were less diminished after the injection of sulindac than were those of the three other prostanoids. Cyclooxygenase inhibition was further confirmed by the marked decreases ($\geq 50\%$) in concentration of prostaglandins E_2, $F_{2\alpha}$ and 6-keto-$F_{1\alpha}$ in the urine samples contrasting with the concentration of thromboxane B_2 which averaged 15 ± 5 and 13 ± 3 ng/ml before and 17.7 ± 7.8, 22.2 ± 11.3 1 h and 11.2 ± 2.8 and 10.3 ± 3.0 ng/ml 2 h after treatment with 5 or 10 mg/kg b.w. sulindac, respectively. Inhibition of cyclooxygenase by sulindac in eight rats not submitted to mechanical pressure changes was in a range similar to that in the rats submitted to mechanical pressure changes (data not shown).

3.6. Interaction between sulindac and proadifen

The effect of proadifen infused in the experimental kidney on the renal vasodilation induced by the low dose of sulindac was investigated in other rats. Baseline mean arterial pressure, renal blood flow and renal vascular resistance averaged, respectively, 117 ± 5, 112 ± 3 and 114 ± 2 mmHg, 7.91 ± 0.46, 8.47 ± 0.60 and 8.75 ± 0.54 ml/min and 15.1 ± 1.3, 13.5 ± 0.9 and 13.2 ± 0.9 mmHg/ml per min in rats infused intrarenally with vehicle ($n = 5$) or with proadifen alone ($n = 7$) or combined with the i.v. injection of 5 mg/kg b.w. sulindac ($n = 6$). These baseline values did not differ between experimental groups. Moreover, mean arterial pressure, renal blood flow and renal vascular resistance did not change significantly with time in the rats infused intrarenally with vehicle. These variables averaged respectively 115 ± 5 mmHg, 7.62 ± 0.35 ml/min and 15.3 ± 1.2 mmHg/ml per min at the end of the experiment. Mean arterial pressure also did not change markedly during the intrarenal infusion of proadifen (end $= 109 \pm 3$ mmHg) while renal blood flow progressively and significantly decreased, averaging 6.25 ± 0.40 ml/min at the end of the experiment (change $= -26\%$; $P < 0.001$). Renal vascular resistance was increased to 18.0 ± 1.5 mmHg/ml per min ($P = 0.003$). Mean arterial pressure and renal blood flow diminished progressively during the combined treatment, averaging, respectively, 98 ± 2 mmHg ($P = 0.002$) and 7.83 ± 0.60 ml/min ($P = 0.009$) at the end of the experiment. Renal vascular resistance then averaged 12.9 ± 1.0 mmHg/ml per min (NS). Fig. 6 summarizes the effects of these different treatments on renal vascular resistance, including for comparison the data for 5 mg/kg b.w. sulindac i.v. illustrated in Fig. 2. Changes in renal vascular resistance from pretreatment values averaged, respectively, 3 ± 4 (vehicle), 32 ± 7 (proadifen), -19 ± 3 (sulindac) and $-3 \pm 2\%$ (proadifen and sulindac) ($P < 0.001$). The change in renal vascular resistance was significantly different from the value with vehicle after proadifen or sulindac alone ($P < 0.05$), but not after the combined treatment. The enhancement in renal vascular resistance after proadifen differed significantly from the reduction in renal vascular resistance after sulindac ($P < 0.05$). Finally, the change in renal vascular resistance was significantly different from that after the combined treatment with proadifen and sulindac after proadifen alone or sulindac alone ($P < 0.05$).

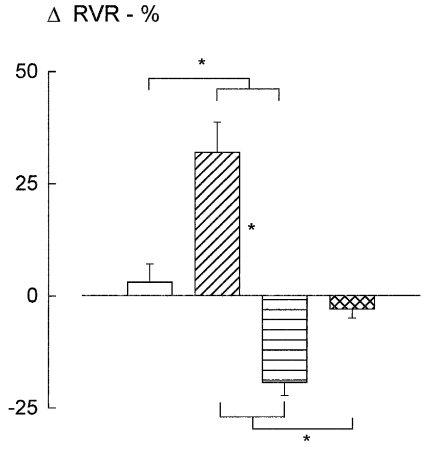

Fig. 6. Percent changes in renal vascular resistance (ΔRVR in %) at the end of the experiment in rats infused intrarenally with vehicle (open bar), 3 mg/kg b.w./h proadifen alone (hatched bar) or combined with 5 mg/kg b.w. sulindac injected i.v. (cross-hatched bar). Percent change in renal vascular resistance 2 h after the i.v. injection of 5 mg/kg b.w. sulindac (horizontal bar) is shown again for comparative purposes (data from Fig. 2). Mean values ± S.E.M. are illustrated. Statistical significance: $P < 0.001$, analysis of variance; * $P < 0.05$, Newman-Keuls test.

4. Discussion

Two findings illustrated the marked differential effects of sulindac on renal hemodynamics in our anesthetized male rats which were kept euvolemic during the surgical preparation. First, significant vasodilation of the kidney occurred after the acute administration of the low dose of sulindac. The renal vasodilation was progressive and was characterized by the concomitant enhancement of renal blood flow and reduction of blood pressure which nevertheless remained within autoregulatory limits. These vascular changes were stabilized 90 min after treatment and no further modifications in renal vascular resistance occurred until the end of the experiment. Conversely, renal vascular resistance did not change significantly in the rats treated with the high dose of sulindac. In these rats, blood pressure decreased to a similar extent and with an identical time course as that in the rats treated with the low dose of sulindac, but renal blood flow did not vary from the control until the end of the experiment. A significant renal vasodilation was also observed under similar experimental

conditions in anesthetized male rats injected intravenously with indomethacin at a dose of 3 mg/kg b.w. (Kramp et al., 1995). Renal blood flow and blood pressure, respectively, increased and decreased simultaneously after this dose of indomethacin. Conversely, no marked renal vasodilator effect occurred in the presence of higher doses of indomethacin (Chevalier et al., 1987; Finn and Arendshorst, 1976; Kramp et al., 1995). In contrast to the effect of sulindac, the renal vasodilation induced by 3 mg/kg b.w. indomethacin did not stabilize during the experiments. Renal blood flow and blood pressure continued to increase or decrease until the end of the experiment, respectively. This difference between effects of 5 mg/kg b.w. sulindac and of 3 mg/kg b.w. indomethacin in the evolution of renal blood flow and blood pressure was not due to variations in our experimental conditions since the strain of rats, the diet and the surgical preparation of the animals were identical. There were no marked differences between basal values for hematocrit, hemodynamic variables, renal function or salt and water excretion. Also, the basal values for plasma renin activity and rates of prostanoid excretion in the urine were in a range similar to those reported from our previous study (Kramp et al., 1995). Moreover, the vasodilator effect was drug-related because renal blood flow and blood pressure did not change spontaneously with time, or with vehicle. Finally, the effects on salt and water retention, although more pronounced with the high dose of sulindac, were comparable to those induced by indomethacin and meclofenamate and resulted in similar decreases of the hematocrit (Kramp et al., 1995).

Second, the relationship between renal blood flow and mean arterial pressure provided further evidence of intrarenal hemodynamic modifications induced by sulindac. In the animals injected with 5 mg/kg b.w. sulindac, the autoregulatory curve of renal blood flow was shifted upward from the control at pressures ≥ 85 mmHg and the autoregulatory plateau tended to be progressively abolished. These changes contrasted markedly with the renal blood flow autoregulatory modifications observed with the high dose of sulindac. Although the autoregulatory curve for renal blood flow was almost similar to the control 1 h after injection of the high dose of sulindac, a definitive leftward shift of the renal blood flow autoregulatory curve from the control occurred 2 h after administration of the drug. At that time, the pressure limits of the autoregulatory plateau were significantly reduced, by approximately 10 mmHg. These changes in the pattern of renal blood flow autoregulation denoted a significant improvement in its efficiency in rats treated with the high dose of sulindac. Similar autoregulatory changes had been reported for rats treated with indomethacin or meclofenamate under identical experimental conditions (Kramp et al., 1995). The factor(s) responsible for the dose-dependent differences in renal hemodynamic changes induced by sulindac are not known. They could reflect some subtle variation in the effects of the two doses of sulindac upon the pathways of arachidonic acid metabolism and/or upon some other vasoactive systems, such as the renin-angiotensin system.

It has been shown that the basal plasma activity of renin was not modified by sulindac in conscious rats on a normal salt diet and given the drug intragastrically at doses as high as 32 mg/kg b.w. (Izumi et al., 1985). Conversely, indomethacin markedly reduced plasma renin activity under similar conditions (Izumi et al., 1985). Sulindac also did not decrease either the basal plasma renin activity in normal human volunteers, or the plasma renin activity of patients with hyperreninemic hyperaldosteronism, again in contrast with the inhibitory effect of indomethacin (Cinotti et al., 1984). In our anesthetized rats, the basal plasma renin activity in systemic arterial or renal venous blood was not decreased by sulindac, irrespective of the dose injected. At the end of the experiment, plasma renin activity even tended to be somewhat enhanced. This change in plasma renin activity was not time-related, nor likely to be due to blood transfusion because plasma renin activity did not constantly increase in the rats treated with vehicle. The reason(s) for the slight increase of plasma renin activity is not known but may be related to stabilisation of the blood pressure reduction which occurred after the injection of sulindac. The interrelationship between plasma renin activity and thromboxane A_2 synthase activity should also be considered, since an inverse relationship between the reduction of thromboxane B_2 excretion and the enhancement of plasma renin activity has been demonstrated when thromboxane A_2 synthase is specifically inhibited or when thromboxane A_2 receptors are specifically blocked (Welch et al., 1989). Nevertheless, it should be pointed out that the enhanced plasma renin activity secondary to a reduction in renal perfusion pressure could be reversed by the high dose of sulindac. Similarly, plasma renin activity increased by a low salt diet could be reduced by 32 mg/kg b.w. sulindac administered intragastrically to conscious rats (Izumi et al., 1985). The effects of high doses of sulindac on enhanced plasma renin activity are thus similar to those previously reported for indomethacin (Chevalier et al., 1987; Izumi et al., 1985). Furthermore, Chevalier et al. (1987) demonstrated that the leftward shift of the renal blood flow autoregulatory curve at subautoregulatory renal perfusion pressures in rats treated with a high dose of indomethacin was related to a diminished plasma renin activity. A similar leftward shift of the renal blood flow autoregulatory curve also occurred at subautoregulatory renal perfusion pressures in our rats treated with the high dose of sulindac. This change in renal blood flow autoregulatory pattern could thus well be related to a reduced plasma renin activity at the subautoregulatory renal perfusion pressure induced by 10 mg/kg b.w. sulindac.

Sulindac was an effective inhibitor of renal prostaglandin synthases under our experimental conditions, since excretion rates and concentrations of prostaglandins E_2, $F_{2\alpha}$ and 6-keto-$F_{1\alpha}$ in urine were markedly diminished irrespective of dose. These results are thus in agreement

with findings with the isolated perfused rat and rabbit kidney, and the conscious rat after intragastric administration of the drug, the anesthetized normal as well as chronic bile duct-ligated dog and in the swine (Miller et al., 1984; Izumi et al., 1985; Zambraski et al., 1984). In contrast, the excretion rates of thromboxane B_2 (not measured in the other studies) were only slightly reduced in our animals while the urinary concentration of thromboxane B_2 was essentially unchanged. These results occurred with each dose of sulindac tested and thus suggest that the activity of thromboxane A_2 synthase was not, or only mildly, depressed by sulindac, at least during the time course of our experiments. Note that indomethacin was much more effective in this regard than sulindac since synthesis of the four prostanoids was inhibited to a similar extent (Kramp et al., 1995). It is also noteworthy that there was similarity in time courses between changes in prostanoid excretion rates and in the hemodynamic variables which stabilized approximately 90 min after the acute treatment with sulindac. Similarity between these temporal profiles suggests resetting of some vasoactive system(s) induced by inhibition of cyclooxygenase activity.

As suggested by McGiff (1991), resetting of a vasoactive system during the acute inhibition of cyclooxygenase can occur within the arachidonic acid cascade itself. Under these conditions, arachidonic acid indeed shifts to the lipoxygenase and cytochrome *P*-450 pathways so that synthesis of arachidonic acid-derived vasoactive products is enhanced in each of these pathways. In the lipoxygenase pathway, some products, such as 15-hydroxyeicosatetraenoic acid, are synthesized in vascular endothelial cells and their production was found to be cyclooxygenase-dependent (Gerritsen, 1996). Likewise, lipoxin A_4, but not lipoxin B_4, is cyclooxygenase-dependent and can induce in vivo vasodilation of the rat kidney (Katoh et al., 1992). Lipoxygenase products could thus be implicated in the renal hemodynamic changes due to sulindac. In this regard, cytochrome *P*-450 arachidonic acid-derived metabolites may prevail because they present 'prominent effects on blood vessels and on ion transport' (McGiff et al., 1996). Our findings that the renal vasodilation induced by the low dose of sulindac was attenuated by the concomitant intrarenal infusion of proadifen are suggestive of, but do not demonstrate, an involvement of the renal cytochrome *P*-450 pathway. Indeed, proadifen, the 'prototypical' inhibitor of the cytochrome *P*-450 pathway (Oyekan et al., 1994), is known to also have other effects. These have been clearly demonstrated by Oyekan et al. (1994) in preconstricted rabbit aortic rings exposed to concentrations of the drug (100 and 200 μM) higher than those (approx. 10 μM) presumably reaching the experimental kidney in our study.

Several mechanisms may be implicated in the relationship between the cyclooxygenase and cytochrome *P*-450 pathways. It has been shown that prostaglandin E_2 exerts an inhibitory effect on the cytochrome *P*-450 arachidonic acid pathway which is reduced or abolished when the activity of cyclooxygenase is inhibited (McGiff, 1991). Therefore, vascular effects of the cytochrome *P*-450 vasoactive products may be further amplified under these conditions (McGiff, 1991). In vivo experiments have also demonstrated that a NSAID can directly interact with several vasoactive products of the cytochrome *P*-450 arachidonic acid pathway and so result in vasodilation of the kidney. Indeed, the intrarenal infusion of 5,6-epoxyeicosatrienoic acid markedly reduces renal vasomotor tone in anesthetized rats treated with ibuprofen (Takahashi et al., 1990) while the renal vasoconstrictor effect of 8,9-epoxyeicosatrienoic acid is reversed by ibuprofen in similarly prepared rats (Katoh et al., 1991). On the other hand, Carroll et al. (1992) found in the isolated perfused rabbit kidney that, not only the vasoactive effects of 5,6-epoxyeicosatrienoic acid, but also those of 20-hydroxyeicosatetraenoic acid were cyclooxygenase-dependent and that these effects could be reversed by indomethacin. Interestingly, these authors (Carroll et al., 1993) also observed that the renal vasodilation induced by 5,6-epoxyeicosatrienoic acid was related to its conversion by cyclooxygenase into 5,6-epoxyprostaglandin E_1, a vasodilator metabolite, and to the release of vasodilator prostaglandins I_2 and E_2. The endothelium-derived hyperpolarizing factor should also be considered in this regard because results of studies done with rings of porcine and bovine coronary arteries suggest that this factor could be a cytochrome *P*-450-derived arachidonic acid metabolite, presumably an epoxide (Hecker et al., 1994). All these findings emphasize the complexity of the interactions between the cyclooxygenase and cytochrome *P*-450 arachidonic acid pathways. Evidently, further investigations with specific inhibitors of the different arachidonic acid pathways must be undertaken to precisely delineate the involvement of each pathway and to unravel the metabolite(s) and mechanism(s) implicated in the renal vasodilation induced by sulindac or other NSAIDs.

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