Contribution of Thromboxane to Renal Resistance Changes in the Isolated Perfused Hydronephrotic Rabbit Kidney

Akiyoshi Kawasaki and Philip Needleman

From the Department of Pharmacology, Washington University Medical School, St. Louis, Missouri

SUMMARY. Thromboxane A2 is not produced in normal rabbit kidneys, but its synthesis is induced in numerous renal pathological states. The presence of this potent vasoconstrictor could readily compromise renal hemodynamics. We found that the thromboxane synthetase inhibitor, OKY-1581 (sodium-3-[4-3-pyridylmethyl[phenyl]-2-methylacrylate) is effective in the perfused hydronephrotic kidney in selectively inhibiting thromboxane production without altering prostaglandin E2 or prostacyclin release. The vasoactive peptides bradykinin and angiotensin II stimulate the hydronephrotic kidney to produce thromboxane A2, which results in a profound renal vasoconstriction which is reversed by pretreatment with OKY-1581. Thus, OKY-1581 provides a powerful tool which can be used to assess the participation of thromboxane in pathophysiological states and to ascertain the therapeutic potential of thromboxane synthetase inhibitors in numerous disease states. (Circ Res 50: 486-490, 1982)

THROMBOXANE A2 (TxA2) biosynthesis has been demonstrated in platelets (Hamberg et al., 1975), lungs (Piper and Vane, 1969), gastrointestinal mucosa (LeDuc and Needleman, 1979), and in the kidney in certain pathological situations (Morrison et al., 1978; Zipsper et al., 1980; Benabe et al., 1980). Several thromboxane synthetase inhibitors (imidazole, N-0164. PG endoperoxide analogs, OKY-1581, and other pyridine derivatives) have been used experimentally to elucidate the role of TxA2 (Needleman et al., 1977a, 1977b; Gorman et al., 1977; Gryglewski et al., 1977; Nijkamp et al., 1977; Miyamoto et al., 1980). Perfusion of rabbit kidneys from animals that had undergone ureter obstruction (hydronephrosis) for several days results in the release of exaggerated levels of PGE2 when stimulated with vasoactive peptides (Nishikawa et al., 1977). As the duration of ex vivo perfusion time increased, the hydronephrotic but not the unobstructed (contralateral) kidney released thromboxane A2 in the renal venous effluent (Morrison et al., 1978; Reingold et al., 1981). The thromboxane A2 was identified by bioassay, radiochemistry, and radioimmunoassay (Morrison et al., 1978; Reingold et al., 1981). The effect of the intrarenal synthesis of thromboxane A2 in the perfused hydronephrotic kidney has not been directly assessed. In the present study, OKY-1581 selectively inhibited renal TxA2 synthesis in the perfused hydronephrotic kidney without altering PGE2 or prostacyclin production. Moreover, OKY-1581 was used to study the participation of TxA2 in the regulation of renal vascular resistance in the perfused hydronephrotic kidney.

Methods

Unilateral ureteral obstruction (hydronephrosis) was performed in New Zealand white rabbits as previously described (Nishikawa et al., 1977). At the time of the experiment, the abdominal cavity was opened and the renal artery was cannulated. The isolated rabbit kidneys were removed from the animals after 7 days of obstruction and perfused at 37°C with oxygenated (95% O2, 5% CO2) Krebs-Henseleit media at a constant flow of 10 ml/min. Changes in perfusion pressure reflected changes in renal resistance. Twenty-five-milliliter aliquots of renal venous effluent were collected for radioimmunoassay before (basal) and after 500-ng bolus injection of the agonist (bradykinin or angiotensin II) stimulation. The thromboxane B2 (TxB2) antiserum had minimal cross-reactivity (<0.02%) with 6-keto-PGF1α, PGE2, and PGF2α (Reingold et al., 1981). The PGE2 antiserum cross-reactivity was as follows: 6-keto-PGF1α, 0.39%; TxB2, 0.03%; and PGF2α, 0.04%. The 6-keto-PGF1α antiserum (kindly supplied by Dr. Richard Fertel, Ohio State University) exhibited the following cross-reactivity: PGE2, 0.57%; TxB2, <0.08%; and PGF2α, 0.14%. The OKY-1581 (sodium-3-[4-3-pyridylmethylphenyl]-2-methylacrylate) was kindly supplied by the Ono Research Institute, Osaka, Japan. Tritium-labeled PGE2, TxB2, and 6-keto-PGF1α were purchased from the New England Nuclear Corporation.

Initiation of the perfusion of the isolated kidney following removal from the rabbit was considered "0" time. The kidneys were stimulated every 30 minutes by bolus injections of agonist (bradykinin or angiotensin II). We previously observed that the amount of PGE2 and thromboxane A2 released by the hydronephrotic kidney in response to bradykinin or angiotensin II was dependent on the duration of the ex vivo perfusion time (Morrison et al., 1978; Reingold et al., 1981). Thus, in the current investigation, we allowed at least 6 hours of perfusion time in order to achieve an adequate level of peptide induced thromboxane release to be detected by our analytical radioimmunoassay of thromboxane B2. The magnitude of the response of the kidney to bradykinin or angiotensin II was not influenced by the presence or absence of injections of agonists during the first 6 hours of perfusion. The levels of PGE2 or thromboxane B2 present in the renal venous effluent of perfused hydronephrotic rabbit kidneys in response to bradykinin or...
angiotensin II during the first 6 hours of perfusion were previously reported (Reingold et al., 1981).

**Results**

The isolated perfused hydronephrotic rabbit kidney exhibited a perfusion time-dependent increase in the release of PGE2 and a time-independent release of prostacyclin (measured as 6-keto-PGF1α; Fig. 1). Infusion of the perfused kidney with OKY-1581 (indicated as the shaded areas on Fig. 1) did not effect the amount of PGE2 or prostacyclin released by bradykinin stimulation. The perfused contralateral unobstructed kidney releases one-twentieth as much PGE2 and 6-keto-PGF1α and undetectable amounts of TxB2 on stimulation with comparable doses of bradykinin (Reingold et al., 1981). However, the isolated hydronephrotic kidney exhibits a perfusion time dependent release of TxB2 upon agonist stimulation (Fig. 2). Before 8 hours of perfusion, the basal levels and bradykinin stimulated thromboxane were low and intrarenal infusion of OKY-1581 did not alter thromboxane release. However, by 9 hours of perfusion, OKY-1581 (0.01 μg/ml) reduced the peptide-stimulated thromboxane level by 58% from 5.2 ± 0.8 to 2.2 ± 0.6 ng/ml. Higher levels of OKY-1581 (e.g., 0.1–1 μg/ml) completely inhibited thromboxane synthesis.

As the perfusion time progresses, the peptide-induced thromboxane release continues to climb sharply; thus, by 12 hours, injection of 500 ng of bradykinin released 250 ng of thromboxane, and this was completely inhibited by 1 μg/ml of OKY-1581 (Fig. 2). Terminating infusion of the inhibitor resulted in rapid recovery of thromboxane release in response to bradykinin.

Having established the effectiveness of OKY-1581 to block thromboxane production in the perfused kidney, we were interested in determining (in 11 experiments) the effect of this agent on the changes in renal resistance induced by the thromboxane release caused by the administration of vasoactive peptides. With increased perfusion time, the renal resistance response to bradykinin bolus injections changed markedly. In six of 11 experiments, the vasodilatory response to bradykinin was decreased and eventually abolished with increasing perfusion time (i.e., 6 hours or longer). In these experiments, infusion of OKY-1581 augmented the decrease in perfusion pressure in response to bradykinin as the agent abolished the thromboxane release. In the remaining five experiments (of 11 experiments), bradykinin caused a reduction in renal resistance early in the experiment (perfusion time, 6 hours or less), but, as time progressed (i.e., 7 hours or more), the peptide produced a profound vasoconstriction associated with the release of high levels of thromboxane into the renal venous effluent (Fig. 3). The increase in pressure produced by bradykinin was reversed to renal vasodilation by treatment with OKY-1581 at 0.1 μg/ml (Figs. 3 and 4). The reduction in renal resistance by OKY-1581 was simultaneous with the suppression of renal thromboxane synthesis. The selectivity of the agent to thromboxane synthetase is exhibited by the lack of effect on PGE2 and prostacyclin production.

Injection of angiotensin II into a perfused hydronephrotic kidney caused a sharp transient rise in renal resistance early (<4-hour perfusion time) in the experiment. As perfusion time progressed, angiotensin II caused a secondary increase in pressure (Fig. 5). Treatment with OKY-1581 at 0.1 μg/ml abolished the exaggerated second phase of increase in perfusion pressure and a vasodilation was observed following a transient increase of perfusion pressure with a reduction of TxB2 production (Figs. 5 and 6). After treatment of the perfused hydronephrotic kidney with indomethacin (1 μg/ml), the bradykinin-induced vasodilatation was attenuated, while the angiotensin II induced vasoconstriction was augmented in both the magnitude and duration of action (not shown). The release of PGE2, 6-keto-PGF1α, and TxB2 was undetectable after the intrarenal indomethacin infusion. Thus, the renal resistance response of the perfused hydronephrotic kidney appears to be a balance of the constricting effects of both the injected intact angiotensin II and the synthesized thromboxane A2 balanced by the renal dilatory influence of PGE2 and prostacyclin.
Discussion

The isolated perfused rabbit kidney is useful as an indication of the synthetic pathway and capacity for intrinsic arachidonate metabolism. It has great advantages over slices and homogenates in allowing direct vascular presentation of agonists to the various renal cell types. Although the Krebs-perfused kidney is a poor model for the study of excretory function, it does provide an intact tissue that maintains receptor recognition which is directly coupled to intrinsic metabolism. Bradykinin and angiotensin are potent stimulators of renal arachidonate metabolism, and these intrinsic metabolites clearly contribute to the status of the renal vascular tone in the perfused rabbit kidney. However, long-term perfusion is required to demonstrate that a bradykinin or angiotensin II injection causes a marked thromboxane release from the hydronephrotic kidney. This perfusion time dependence is specifically tied to the response of the hydronephrotic kidney to bradykinin or angiotensin II and not to the thromboxane synthetase capacity. We have previously demonstrated in the rabbit that microsomes prepared from an unperfused ureter-obstructed kidney, but not the contralateral unobstructed kidney, possess substantial thromboxane synthetase activity (Morrison et al., 1978). Thus, although there is little question about the thromboxane synthetic capacity attendant to ureter obstruction, neither the in vivo agonists that activate renal thromboxane production nor plasma or urine levels of metabolites have been reported. The current investigation demonstrates the potential for renal thromboxane production and its ability to elicit a profound renal vasoconstriction, and this work with the thromboxane synthetase inhibitor therefore provides a rational strategy for manipulation of renal resistance in ureter obstruction. Thromboxane production is also associated with the renal damage observed in rabbits with renal venous constriction (Zipser et al., 1980), and glycerol-induced acute tubular necrosis (Benabe et al., 1980). The fact that such different circumstances induce the synthesis of a powerful vasoconstrictor, which is not detectable in normal rabbit kidneys, suggests that the unmasking of this pathway may be a common renal compensatory mechanism.

OKY-1581 significantly inhibits the bradykinin-stimulated production of thromboxane \( B_2 \) at 0.01 μg/ml, whereas PGE\(_2\) and 6-keto-PGF\(_{1α}\) production were not affected at 1 μg/ml (Figs. 1 and 2). TXA\(_2\) produced in response to bradykinin or angiotensin II caused renal vasoconstriction and modified the effects of both peptides on renal vascular resistance. In such
BRADYKININ 500ng

FIGURE 4. Reversal by inhibition of thromboxane synthetase of the bradykinin-induced renal vasoconstriction of the perfused hydronephrotic kidney. In the five experiments in which bradykinin elicited a renal vasoconstriction, the immunoassayable level of thromboxane B2 released in response to bradykinin (500-ng bolus) present in the venous effluents taken 30 minutes before, during, and 30 minutes after infusion of OKY-1581 (0.1 µg/ml) was 4.6 ± 0.7 ng/ml, 0.7 ± 0.3, and 4.9 ± 1.3 ng/ml, respectively (n = 5). The level of PGE2 (107 ± 18 ng/ml n = 5) or 6-keto-PGF1α (36 ± 14 ng/ml) was not changed by the OKY-1581 infusion.

ANGIOTENSIN II 500ng

FIGURE 5. A representative experiment showing the effect of OKY-1581 on changes in perfusion pressure induced by angiotensin II (500 ng) injection and release of thromboxane B2 (TXB2), prostaglandin E2 (PGE2), and 6-keto-PGF1α (6-keto-PGF1α) in rabbit isolated hydronephrotic kidney. The exaggerated second phase of increase in perfusion pressure caused by the bolus injection of angiotensin II was abolished by the infusion of the OKY-1581. These are the data from one experiment; the grouped data are presented in Figure 6.

FIGURE 6. Quantitation of the effect of the thromboxane synthetase inhibitor on the release of renal arachidonate metabolites induced by angiotensin II in the perfused hydronephrotic kidney. The angiotensin II-induced TXB2 levels present before, during, and after OKY-1581 were 4.4 ± 1.5 ng/ml 0.6 ± 1, and 2.7 ± 0.9 ng/ml, respectively (n = 5). The PGE2 (75 ± 11) and 6-keto-PGF1α (49 ± 8 ng/ml) levels were unchanged by the thromboxane synthetase inhibitor.

circumstances, OKY-1581 unmasked the vasodilatory effects of the massive release of PGE2. Thus, the small amount of thromboxane A2 was able to overcome the vasodilatory effect of the injected bradykinin and the concomitantly released PGE2 and PGI2. Similarly, treatment with OKY-1581 abolished the second phase in the increase of perfusion pressure induced by angiotensin II, and a vasodilation was observed following a transient increase in perfusion pressure. In contrast to OKY-1581, indomethacin treatment attenuated the vasodilation of bradykinin and augmented the vasoconstriction of angiotensin II (not shown). These results demonstrate that PGs and TXA2 produced by the injection of bradykinin and angiotensin II contribute to the effects of both peptides on renal vascular resistance. On the other hand, whereas imidazole is effective in blocking microsomal thromboxane synthetase, imidazole was ineffective in inhibiting the bradykinin- or angiotensin-induced release of thromboxane A2 from the perfused hydronephrotic kidney (not shown).
The renal resistance vessels (afferent and efferent glomerular arterioles) are cortical, and this area receives the highest fraction of blood flow (Thorburn et al., 1963). We previously demonstrated that cortical microsomes obtained from hydronephrotic kidneys (Needleman et al., 1979) and renal venous constricted kidneys (Zipser et al., 1980) possesses more cyclo-oxygenase activity than microsomes prepared from contralateral (unobstructed) or normal kidneys. The arachidonate metabolites produced in the cortex of pathological kidneys appear to be produced, at least in part, at a site that has ready access to modulate renal resistance. The validation in our experiments of the utility of OKY-1581 as a selective thromboxane synthetase inhibitor in the intact kidney should now provide a tool to discriminate clearly the participation of thromboxone in various renal disease states. Very recently (Tyler et al., 1981), a previously unreported agent, UK-37,248-01 was administered orally to patients and found to reduce plasma thromboxane B$_2$ levels and inhibit aggregation. OKY-1581 was shown earlier to be effective in the circulation of intact patients and found to reduce plasma thromboxane B$_2$ levels and inhibit aggregation. OKY-1581 was shown earlier to be effective in the circulation of intact rabbits in preventing platelet thromboxone production (Miyamoto et al., 1980). Thus, OKY-1581, probably as well as UK-37,248-01, provides a powerful tool for assessing the therapeutic potential of thromboxone synthetase inhibitors in numerous disease states. Some other obvious candidate situations where inhibition of intrinsic thromboxone production may be beneficial include: coronary and cerebral thrombosis and vasospasms, ulcers, arthritis, inflammation, and bronchoconstriction.

References


LeDuc LE, Needleman P (1979) Regional localization of prostacyclin and thromboxane synthesis in dog stomach and intestinal tract. J Pharmacol Exp Ther 211: 181-188


Contribution of thromboxane to renal resistance changes in the isolated perfused hydronephrotic rabbit kidney.
A Kawasaki and P Needleman

Circ Res. 1982;50:486-490
doi: 10.1161/01.RES.50.4.486

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/50/4/486