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Indomethacin Increases Renal Vascular Resistance after Adrenergic Interruption in the Dog (40628)[1]

INA C. EHRHART, GARY C. BOND, AND JACK M. GINSBURG

Department of Physiology, Medical College of Georgia, Augusta, Georgia 30902

Prostaglandin (PG) synthetase inhibition variably affects renal hemodynamics. For example, several investigators (1–4) have demonstrated that indomethacin, a drug inhibiting the conversion of arachidonic acid to PGs, produces a marked increase in renal vascular resistance (RVR) and a decrease in renal blood flow (RBF) in anesthetized and surgically stressed dogs. In contrast, other investigators (5–7) have shown that indomethacin does not alter RVR and RBF in the nonstressed dog. Zins (6) hypothesized that the different results could be related to the level of autonomic activity existing prior to indomethacin treatment. In the stressed dog the high level of autonomic activity may lead to increased production of renal PGs which oppose adrenergic vasoconstrictor effects on renal vascular tone. Thus, PG synthetase inhibition in the stressed dog could result in increased RVR and decreased RBF. In contrast, autonomic activity and, therefore, PG levels, may be low in the nonstressed animal. Thus indomethacin infusion in these animals would have little effect on renal hemodynamics. The experiments reported here were conducted to test this hypothesis. The renal vascular response to the PG synthetase inhibitor, indomethacin, was studied in the anesthetized, surgically stressed dog before and after α-adrenergic blockade or renal denervation.

Materials and methods. Twenty-seven mongrel dogs (14 to 26 kg) were anesthetized with pentobarbital sodium, 30 mg/kg i.v. Ventilation was maintained at a constant frequency and tidal volume. Either kidney was approached through a flank incision and an electromagnetic flow transducer (Biotronex) placed around the renal artery. Electrically meaned femoral arterial blood pressure (MAP) and RBF were recorded on a Beckman Model R411 oscillograph. Zero flow was determined by occluding the renal artery distal to the flow probe. The kidney studied was removed and weighed at the end of the experiment.

Two series of experiments were performed. The effects of α-adrenergic blockade on the renal vascular response to indomethacin was studied in Series I. Nine control dogs received only indomethacin while 11 dogs received indomethacin after α-blockade. Indomethacin 0.1–0.2 mg/(kg $\times$ min) was infused over 30 min through a 20-gauge needle positioned directly in the renal artery distal to the flow probe. The total dose was dissolved in 100 ml of 22 m*M* sodium bicarbonate–isotonic saline (pH 8.3). This dosage of indomethacin exceeds that reported to inhibit prostaglandin synthesis (2). Arachidonic acid, 10 mg in 1 ml ethanol, was given i.v. as a bolus before and 30 min following indomethacin. Attenuation of the expected decrease in MAP due to arachidonic acid was interpreted as an indication of inhibition of PG synthesis. α-Adrenergic blockade was induced by phenoxybenzamine hydrochloride (Dibenzyline) at a dose of 1.6–2.3 μg/(kg $\times$ min), $n = 5$, or a dose of 50 μg/(kg $\times$ min), $n = 6$, both doses given into the renal artery over 20 min. The total dose was diluted with 20 ml normal saline. Phenoxybenzamine infusion was completed at least 20 min prior to infusion of indomethacin. Efficacy of α-blockade was tested by injecting a 1 or 4 μg bolus of L-norepinephrine bitartrate (Levophed) into the renal artery before and after phenoxybenzamine. Norepinephrine, as the base, was diluted in 1 ml normal saline. The criterion for α-blockade was a marked attenuation of the expected decrease in RBF in response to norepinephrine. To test the response to the indomethacin vehicle, 13 Series I dogs received a bicarbonate–saline infusion prior to the indomethacin infusion.

The effect of renal denervation on the renal vascular response to indomethacin was stud-

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ied in Series II. In seven dogs the response to indomethacin in an innervated kidney was compared to the response in the same kidney 5–6 days after denervation. Observations in the innervated kidney were made identically to those in Series I except that aseptic conditions were maintained and indomethacin was infused into a peripheral vein to eliminate the possibility of postoperative hemorrhage from the renal artery. The exposed kidney was then decapsulated and denervated by stripping the renal artery, vein, and proximal ureter. Decapsulation facilitated the denervation. The artery and vein were painted with 8% phenol. Penicillin G combined with dihydrostreptomyocin (Combiotic) was administered i.m. for 4 days following surgery.

After data was collected from the denervated kidney, both the denervated kidney and the contralateral innervated kidney were rapidly removed and weighed. Slices were frozen in liquid nitrogen and stored at −180° until assayed for norepinephrine content by the spectrophotofluorometric method of Ciarlone (8).

In all dogs RBF reached a low value by the midpoint of the indomethacin infusion (approx 17 min) and remained at this level throughout and for at least 15 min following the infusion (Fig. 1). Average MAP and mean RBF were obtained by dividing this steady-state time interval into the planimetrically determined area under the pressure–time curve or the flow–time curve over this interval. Average MAP and RBF were used to calculate RVR. Statistical comparison of responses to pharmacologic interventions were made within groups and between groups using Student's paired and nonpaired t tests, respectively. Because preindomethacin values differed significantly between the α-blocked and control groups, experimental data were expressed in percentage of preindomethacin values. P values <0.05 are considered indicative of statistical significance. Data are expressed as mean ± SEM.

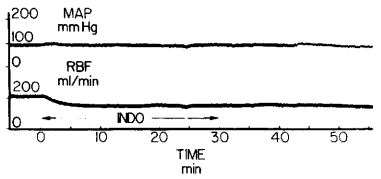

FIG. 1. Representative experimental record of the time course of mean arterial blood pressure (MAP) and renal blood flow (RBF) changes associated with indomethacin (INDO) infusion. Arrows indicate the beginning and end of infusion.

Results. In Series I, one of two different doses of phenoxybenzamine was used to produce blockade. Renal arterial administration of 1 or 4 μg of norepinephrine reduced RBF 31 or 78%, respectively ($P < 0.01$), prior to phenoxybenzamine, but had no significant effect on flow ($P > 0.10$) following α-blockade with phenoxybenzamine. Since there were no statistical differences in the responses to indomethacin for dogs receiving 1.6–2.3 μg/(kg × min) and those dogs receiving 50 μg/(kg × min) of phenoxybenzamine, these data were combined to make up the α-blocked group.

Figure 2A shows the effect of indomethacin infusion on RVR, RBF, and MAP in the α-blocked and the control groups of Series I. Prior to indomethacin, RVR in the α-blocked dogs was 0.28 ± 0.04 as compared to 0.44 ± 0.06 (mm Hg·min·100 g)/ml in the control dogs ($P < 0.05$). In response to indomethacin, RVR increased an average of 22% in both the α-blocked group ($P < 0.001$) and the control group ($P < 0.005$) while RBF decreased an average of 18% in the α-blocked ($P < 0.005$) and 16% in the control ($P < 0.005$). The percentage changes in RVR and RBF did not differ between the two groups of dogs ($P > 0.50$). MAP was unaffected by indomethacin in either group ($P > 0.50$). Indomethacin-free sodium bicarbonate–saline solution had no effect on measured variables.

Responses to indomethacin infusion before and 5–6 days following renal denervation, Series II, are shown in Fig. 2B. Although preindomethacin RVR in the intact Series II kidney appears higher than RVR in the Series I control kidney both before and after indomethacin, the differences were not significant. Indomethacin increased RVR an average of 23% before ($P < 0.05$) and 35% after ($P < 0.005$) renal denervation and decreased RBF an average of 19% before ($P < 0.01$) and 25% following denervation ($P < 0.025$). The changes in RVR and RBF associated with indomethacin did not differ between the innervated and denervated kidneys ($P > 0.20$). MAP was not changed ($P > 0.10$) by indo-

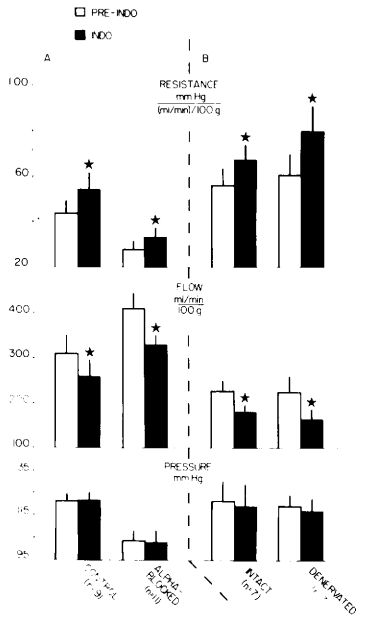

FIG. 2. Renal vascular resistance, renal blood flow, and mean arterial pressure responses to indomethacin 0.1–0.2 mg/(kg/min) × 30 min. (A.) Series I: Responses to renal arterial infusion of indomethacin in α-blocked and in control dogs. (B.) Series II: Responses to intravenous infusion of indomethacin in the same kidneys before and following renal denervation, n = number of dogs. The star indicates significant difference from preindomethacin value ($P < 0.05$).

methacin.

In seven experiments, denervation reduced renal norepinephrine content ($P < 0.001$) from 0.703 ± 0.052 in the innervated kidney to 0.136 ± 0.008 μg/g wet wt in the contralateral denervated kidney. Recovery of norepinephrine, estimated by use of an internal standard was 67.1% ± 2.01. In nine trials in six dogs (three trials prior to and six following renal denervation), arachidonic acid decreased MAP 24% ($P < 0.001$) prior to indomethacin but did not change MAP ($P > 0.10$) following indomethacin infusion.

Discussion. Our studies do not support the hypothesis that indomethacin increases RVR by eliminating PG modulation of sympathetic vasoconstrictor activity in the anesthetized, surgically stressed dog. α-Adrenergic blockade, with doses of phenoxybenzamine sufficient to eliminate the decrease in RBF in response to norepinephrine, did not diminish the increase in RVR in response to indomethacin. Furthermore, surgical renal denervation failed to attenuate the renal vasoconstrictor response to indomethacin although it reduced the norepinephrine content of the kidney to ⅕ that of the innervated control. Indomethacin eliminated the decrease in MAP seen in response to arachidonic acid, a PG precursor, thus providing evidence for inhibition of PG synthesis. The validity of the hypothesis that renal adrenergic activity is modulated by PGs would seem to depend upon verification of PG secretion as a function of renal sympathetic activity. Although PG release in response to renal sympathetic stimulation has been reported in several species (9, 10), McGiff *et al.* (11) reported no increase in canine renal vein PGE with renal nerve stimulation.

Our observation that the effect of indomethacin on RVR was not altered by adrenergic interruption is in agreement with the finding of Chapnick *et al.* (12) that α-adrenergic blockade does not attenuate the rise in RVR in response to indomethacin in the feline kidney. Their studies, like ours, were performed in presumably normovolemic animals. The data of Henrich *et al.* (13) indicates that indomethacin is equally effective in reducing RBF in both denervated and innervated kidneys of anesthetized, surgically stressed dogs under normovolemic conditions. However, in the same study renal denervation attenuated the reduction in RBF associated with hypotensive hemorrhage in the indomethacin-treated dog. This observation suggests an interaction between renal nerve and PG effects on renal hemodynamic responses to hypotensive hemorrhage.

The increase in RVR associated with indomethacin might be related to an interaction between PG synthesis and renal vasoconstriction caused by angiotensin II. There is evidence that angiotensin II releases PGs which diminish the renal vasoconstrictor effect of this pressor hormone (14). Terragno *et al.* (7) reported that plasma renin activity is highly correlated with renal PGE-like substances in renal venous blood of conscious or surgically stressed dogs. Furthermore, interruption of the renin–angiotensin system diminishes the increase in RVR associated with inhibition of prostaglandin synthesis in the normovolemic animal (12, 15). Recently, Henrich (16) re-

ported that antagonism of the renin–angiotensin system coupled with renal denervation protects against the renal vasoconstriction associated with hypotensive hemorrhage following PG synthetase inhibition.

Our study shows that the effect of indomethacin on RVR in the normovolemic dog is independent of the adrenergic nervous system. We find no evidence for endogenous PGs as important modulators of the adrenergic nervous system in the control of RBF in the normovolemic dog. However, our findings, along with those cited, do not rule out the possibility that indomethacin has a direct effect on RVR or that indomethacin increases RVR by eliminating PG modulation of the renin–angiotensin system.

Summary. The effect of adrenergic interruption on the renal vascular resistance (RVR) increase associated with the prostaglandin (PG) synthetase inhibitor, indomethacin, was studied in anesthetized, surgically stressed dogs. α-Adrenergic blockade was produced with phenoxybenzamine. In response to indomethacin, neither the increase in RVR nor the decrease in renal blood flow (RBF) differed between the α-blocked (n = 11) and the control dogs (n = 9). In a separate series of experiments, the effect of renal denervation on the renal vascular response to indomethacin was examined in the same kidney before and 5–6 days after that kidney was surgically denervated (n = 7). The indomethacin-associated increase in RVR and decrease in RBF did not differ in the intact and denervated kidneys. Under conditions of anesthesia and surgical stress in normovolemic dogs, neither α-adrenergic blockade nor renal denervation changed the renal hemodynamic response to indomethacin. We, therefore, conclude that under these conditions the indomethacin-induced increase in RVR is not mediated by elimination of PG modulation of renal sympathetic vasoconstrictor activity.

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1. Lonigro, A. J., Itskovitz, H. D., Crowshaw, K., and McGiff, J. C., Circ. Res. **32,** 712 (1973).
2. Aiken, J. W., and Vane, J. R., J. Pharmacol. Exp. Ther. **184,** 678 (1973).
3. Owen, T. L., Ehrhart, I. C., Weidner, W. J., Scott, J. B., and Haddy, F. J., Proc. Soc. Exp. Biol. Med. **149,** 871 (1975).
4. Beilin, L. J., and Bhattacharya, J., J. Physiol. **271,** 625 (1977).
5. Swain, J. A., Heyndrick, C. R., Boettcher, D. H., and Vatner, S. F., Amer. J. Physiol. **229,** 826 (1975).
6. Zins, G. R., Amer. J. Med. **58,** 14 (1975).
7. Terragno, N. A., Terragno, D. A., and McGiff, J. C., Circ. Res. **40,** 590 (1977).
8. Ciarlone, A. E., Microchem. J. **21,** 349 (1976).
9. Davis, H. A., and Horton, E. W., Brit. J. Pharmacol. **46,** 658 (1972).
10. Dunham, E. W., and Zimmerman, B. G., Amer. J. Physiol. **219,** 1279 (1970).
11. McGiff, J. C., Crowshaw, K., Terragno, N. A., Malik, K. U., and Lonigro, A. J., Clin. Sci. **42,** 223 (1972).
12. Chapnick, B. M., Paustian, P. W., Feigen, L. P., Joiner, P. D., Hyman, A. L., and Kadowitz, P. J., Circ. Res. **40,** 348 (1977).
13. Henrich, W. L., Anderson, R. J., Berns, A. S., McDonald, K. M., Paulsen, P. J., Berl, T., and Schrier, R. W., J. Clin. Invest. **61,** 744 (1978).
14. McGiff, J. C., Crowshaw, K., Terragno, N. A., and Lonigro, A. J., Circ. Res. **27** (Suppl. I), I–121 (1970).
15. Satoh, S., and Zimmerman, B. G., Circ. Res. **36–37** (Suppl. I), I–89 (1975).
16. Henrich, W. L., Berl, T., McDonald, K. M., Anderson, R. J., and Schrier, R. W., Amer. J. Physiol. **235,** F-46 (1978).

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