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Effects of Indomethacin on Local Blood Flow Regulation in Canine Heart and Kidney[1] (38916)

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The study of prostaglandins as regulatory agents in the cardiovascular system has in many instances involved the use of indomethacin, a potent inhibitor of prostaglandin synthetase (1, 2). The infusion of indomethacin into the canine heart has been reported in a preliminary communication to attenuate the hyperemias of coronary occlusion and hypoxia under certain conditions (3). Intravenous injection of indomethacin caused increased vascular resistance in the dog kidney (4–6), and attenuated renal autoregulation in one study (4) but not in another (6). In this study, we infused indomethacin into the coronary arteries of closed-chest animals to determine its effect on coronary reactive hyperemia. We also studied the effect of close intra-arterial infusion of indomethacin on renal blood flow, autoregulation and reactive hyperemia.

Methods. Two series of experiments were performed. To study the coronary vascular bed, 14 mongrel dogs of either sex (22–30 kg) were sedated with 30 mg morphine sulfate and anesthetized with urethane (500 mg/kg) and choralose (75 mg/kg). The animals were anticoagulated with 20,000 units heparin, and were mechanically ventilated with room air at a rate and volume which maintained arterial pH about 7.4. A catheter was inserted into the aorta via a femoral artery for the measurement of pressure. One lead of the electrocardiogram was recorded to determine heart rate and detect arrythmias. Coronary blood flow was measured in these closed-chest animals with a velocity-sensitive cathetertip flowmeter (7). The flowmeter catheter was passed retrograde through the right carotid artery and placed in the aorta with the curved tip inserted into the orifice of the left coronary artery and then wedged into either the left anterior descending or circumflex artery. It was determined that the flowmeter tip was wedged in a coronary branch by the reactive hyperemia following a few seconds of complete ischemia produced with an occlusive sheath on the flowmeter. Coronary flow, aortic pressure and the electrocardiogram were recorded on an oscillograph. Planimetry was used to compute coronary flow. Flowmeter position was confirmed at autopsy.

Indomethacin was infused directly into the coronary arterial bed via an infusion port on the flowmeter. In eight experiments, 90 mg of indomethacin was dissolved in 100 ml of a NaOH-saline solution (final pH = 10.2–10.4). This solution was immediately infused over a period of 30–60 min. Blood pressure, coronary flow and heart rate were not affected by the infusion. Hyperemic flow was measured following relief of 15 sec occlusions before and at 15 min intervals for at least 1 hr after indomethacin. Hyperemic flow was unaffected. However, since spectrophotometric analysis, visual observation and consultation with the pharmaceutical firm (personal communication, F. A. Bacher, Director, Pharmaceutical Analysis, Merck, Sharp and Dohme, West Point, PA) indicated that the half life of indomethacin at pH 10.4 is about 20 min, it was deemed necessary that another series of animals be studied. In this series of six animals, 90 mg of the drug was dissolved in 5 ml ethanol and this was brought to 100 ml with a phosphate-buffered saline solution

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(final pH = 7.2). The integrity of the drug in this solution was confirmed for up to 48 hr by spectrophotometric analysis. Four animals received 90 mg in 1 hr. The remaining two animals received 200 mg in two hours. The response to 5 and 15 sec coronary occlusions were observed before and at 30 min intervals for 2 hr following the initiation of the indomethacin infusion.

To study the renal vasculature, 28 mongrel dogs of either sex (10–15 kg) were anesthetized with pentobarbital, heparinized and mechanically ventilated. In eleven natural flow experiments, the left renal vein was cannulated while the renal artery and nerves remained intact. Renal blood flow was returned to the animal via the femoral vein. Renal outflow was measured with an electromagnetic flowmeter (Biotronex Laboratory, Model 610, BLC-2048-E04 cannulating probe) in the venous outflow tubing. A small gauge needle was inserted into the renal artery to measure renal perfusion pressure and to infuse indomethacin. An adjustable clamp was placed around the renal artery to facilitate reduction of renal artery pressure or renal artery occlusion. In this series, autoregulation was studied only at arterial pressures below normal. Reactive hyperemia was observed following a 90 second occlusion of the renal artery. In six animals, observations were made before, immediately after and 1 hr after a 30 min intra-arterial infusion of 90 mg indomethacin, dissolved in 100 ml of bicarbonate-buffered saline (pH 7.4; 22 m*M* $NaHCO_3$). Indomethacin was shown to be stable in this solution for at least 48 hr. In five animals observations were made before, immediately after and 1 hr after a 30 min intra-arterial infusion of buffered saline (no indomethacin).

In another series of nine experiments, flow to the kidney was controlled while measuring the perfusion pressure. The renal nerves were severed and the renal artery was cannulated and perfused at various flow rates with a precalibrated Sigmamotor pump. Perfusion pressure was also measured before, during and after shutting off the pump for various periods of time. Following control observations, indomethacin (90 mg in 100 ml bicarbonate-buffered saline) was infused over a 60-min period directly into the renal perfusion cannula, and the maneuvers repeated.

In a third series of 8 animals, renal perfusion was regulated with an electronic controller (Leeds and Northrup, Model 420 type P) connected to a servo-pump (Holter RE 161) which perfused the kidney. The pump's speed was recorded and calibrated as renal blood flow. Autoregulation was studied by altering renal perfusion pressures over a range of 50–200 mmHg, in step increments of 25 mmHg, and recording flow at each pressure. Reactive hyperemia was measured following a 90 sec period of no flow. Observations were made before, immediately after and one hour after a 60 min renal artery infusion of 90 mg indomethacin dissolved in 100 ml of bicarbonate-buffered saline.

Results. Coronary vascular bed. Heart rate and systemic arterial pressure did not change significantly during the course of the experiments. Table I compares the hyperemic responses in the coronary vascular bed to the 5 sec occlusions before and after indomethacin. It is obvious that neither the flow at 5 sec intervals nor the mean flow for the entire period of hyperemia (last column) was altered by indomethacin. The response 30 min after initiation of infusion (not shown) also did not differ from that during the control period. It can also be seen that indomethacin, *per se*, did not alter coronary flow (first column) in these six closed-chest animals. Fifteen second occlusions were also studied in these experiments. As with the 5 sec occlusions, the hyperemia following release of occlusion was unaffected by indomethacin.

Renal vascular bed. Table II presents the average effects of indomethacin on control flow, autoregulation and reactive hyperemia in the six kidneys studied at natural flow. Reduction of renal arterial pressure produced a significant decrease in renal vascular resistance. Following release of a complete occlusion for 90 sec, there was a significant increase in renal blood flow. Indomethacin significantly increased renal vascular resistance and decreased blood flow. Reduction of perfusion pressure produced the same change in resistance, but the reactive

TABLE I. EFFECT OF INDOMETHACIN ON REACTIVE HYPEREMIA IN THE CORONARY VASCULAR BED OF THE INTACT DOG.[a]

Time (min)[b]	Coronary blood flow (ml/min ± SE)						
	Before	Following release of 5 sec occlusion					
		0–5s[c]	5–10s	10–15s	15–20s	20–25s	25–30s	0–30s
−5	34 ± 4	114 ± 19	74 ± 16	50 ± 11	42 ± 8	38 ± 5	37 ± 6	59 ± 11
60	32 ± 5	105 ± 16	74 ± 10	45 ± 7	39 ± 6	35 ± 5	34 ± 5	55 ± 7
90	36 ± 5	103 ± 11	77 ± 12	48 ± 10	40 ± 7	38 ± 6	38 ± 6	57 ± 9
120	36 ± 4	104 ± 14	83 ± 9	52 ± 7	42 ± 5	39 ± 4	37 ± 5	60 ± 6

[a] $n = 6$.
[b] Time before and after starting indomethacin infusion into coronary artery.
[c] s = seconds. Flow is the average flow over the time interval indicated.

TABLE II. EFFECTS OF INDOMETHACIN ON AUTOREGULATION AND REACTIVE HYPEREMIA IN THE NATURALLY PERFUSED RENAL VASCULAR BED OF THE DOG.[a]

Time	Autoregulation				Reactive hyperemia[c]		
	Renal artery pressure		Renal resistance		Renal Venous Flow		
	Control	Constrict	Control	Constrict	Control	Peak	Excess
	mm Hg	mm Hg	PRU[b]	PRU	ml/min	ml/min	ml
Before indomethacin	108	81[e]	0.60	0.44[e]	175	226[e]	94
Immediately after	113	76[e]	0.76[d]	0.53[d,e]	145[e]	183[d,e]	36[d]
One hour after	117	80[e]	0.77[d]	0.60[d,e]	144[d]	178[d,e]	34[d]

[a] $n = 6$.
[b] mmHg/ml min^{-1}.
[c] In response to a 90 sec occlusion of the renal artery.
[d] $P < 0.05$ relative to value before indomethacin.
[e] $P < 0.05$ relative to control value.

hyperemic flow following occlusion was diminished. In five control experiments (buffered saline alone infused), renal resistance remained constant, and autoregulation and reactive hyperemia were not altered by the saline infusion.

When flow was the controlled variable, the responses were more difficult to interpret. As observed previously (8, 9), perfusion pressures were stable at low constant flow, but gradually increased at high constant flow, sometimes to levels which can cause renal hemorrhage. Moreover, as also observed previously (10), at the low flow rates required for stability, stopping flow for a period of time was, on restarting flow, just as likely to result in reactive constriction as reactive dilation. This was especially true at low (less than 80 mmHg) perfusion pressures. However, indomethacin increased renal resistance from a preinfusion value of 0.99 (± 0.09 SE) to 2.47 (± 0.43 SE) thirty minutes after infusion had begun ($n = 9$; $P < 0.1$) and autoregulation still occurred following indomethacin, as evidenced by large increases in resistance when flow was elevated.

Figure 1 shows tracings from a typical experiment in which renal perfusion pressure was adjusted over the range 50–200 mmHg. The record shows that, in the steady state, there was good autoregulation of flow over the range 100–200 mmHg. This was true both before and immediately after indomethacin infusion, although flow was reduced at all pressures after indomethacin. In seven of eight dogs, steady-state flow was less affected by decreasing pressures than

RENAL AUTOREGULATION AND REACTIVE HYPEREMIA

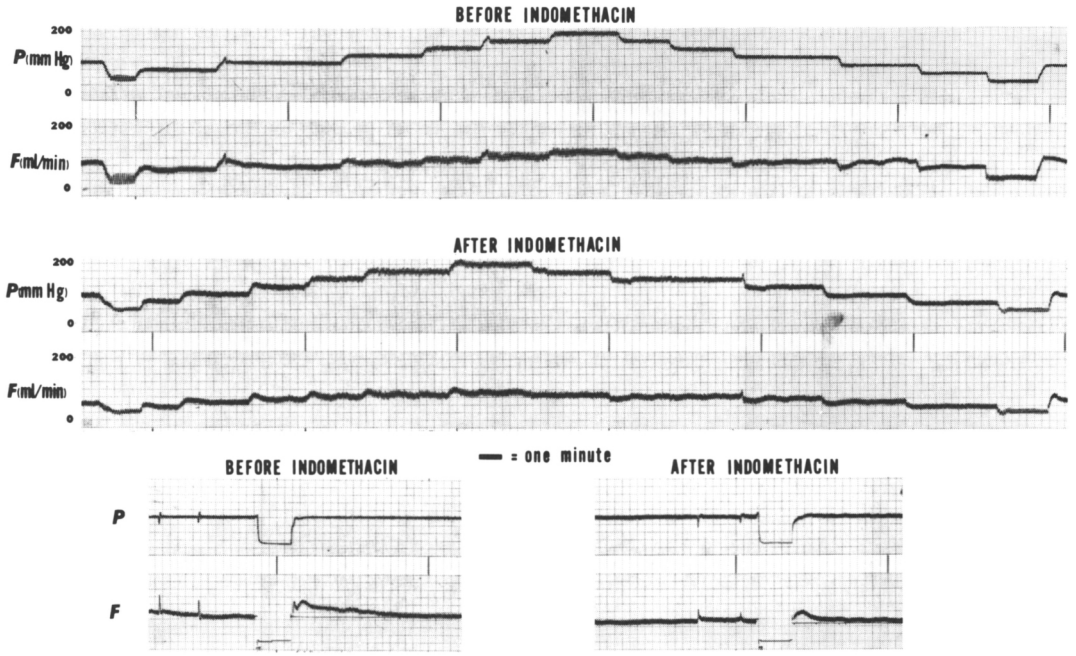

FIG. 1. Typical flow responses to changes in perfusion pressure and to total ischemia before and immediately after indomethacin.

by increasing pressures, resulting in a "hysteresis" in the pressure-flow curves. This is apparent in Fig. 2, which presents average data. The hysteresis, suggesting a greater autoregulatory ability as pressure is decreased, was absent after indomethacin infusion. The averaged data also shows that renal blood flow was decreased at all pressures after indomethacin infusion, persisting for at least 1 hr, and that the slopes of the curves on increasing pressure were unaffected.

Reactive hyperemia, as measured by either mean blood flow, duration of hyperemia, or total excess blood flow during the hyperemia, was significantly attenuated for at least an hour following indomethacin infusion (Figs. 1 and 3).

Discussion. The results of these studies suggest that (a), intracoronary infusion of indomethacin does not affect reactive hyperemia in the coronary vascular bed and

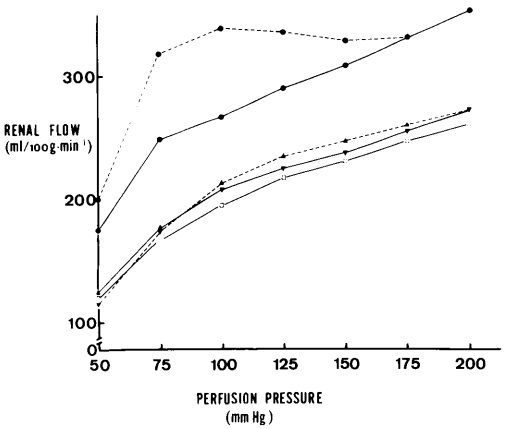

FIG. 2. Average pressure-flow curves before, immediately after, and 1 hr after indomethacin infusion. ● = before indomethacin, △ = immediately after indomethacin and □ = 1 hr after indomethacin. Solid line indicates stepwise increase in pressure. Broken line indicates stepwise decrease in pressure. Pressure sequence as in Fig. 1.

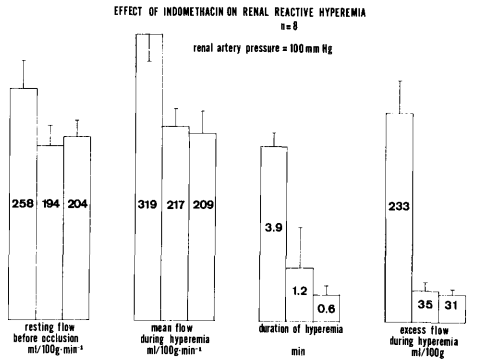

FIG. 3. Effect of indomethacin on resting renal flow and reactive hyperemia. For each parameter, left bar represents measurement before indomethacin; middle bar, immediately after right bar, 1 hr after.

(b), the kidney still exhibits autoregulation and reactive hyperemia following indomethacin infusion into the renal artery but that the reactive hyperemia is attenuated and the autoregulatory response to decreasing pressures is modified.

The reactive hyperemia occurring after a 5 or 15 sec coronary occlusion was unaffected by indomethacin infusion in these studies. This is in contrast to the findings of Kent *et al.* reported in a preliminary communication (3). They found that the reactive hyperemia following 10, 15 or 20 sec coronary occlusions is reduced by blockage of prostaglandin synthetase with indomethacin. It seems unlikely that in our study the concentration of indomethacin in the blood perfusing the coronary vasculature was too low to inhibit prostaglandin synthesis. The calculated concentration for most experiments was approximately 120 μg indomethacin per ml of blood for 30 min (a total dose of 3–4 mg/kg body weight). Although comparable data for intact canine heart could not be found in the literature, indomethacin concentrations of less than 25 μg/ml totally abolish the outflow of prostaglandins from isolated perfused rabbit hearts (11). A major difference between our study and that of Kent *et al.* relates to the preparation; they used isolated heart–lung preparations and open-chest animals. We used closed-chest animals. In our animals, indomethacin did not affect resting coronary flow. In their heart–lung preparations, coronary flow and coronary sinus efflux of prostaglandin E gradually increased with time and both were reduced by indomethacin. Since prostaglandin synthesis is increased by a number of mechanical and chemical stimuli, the surgical preparation may be a significant determinant of prostaglandin production and the effects of subsequent inhibition of prostaglandin synthesis on resting flow and possibly on reactive hyperemia. Our studies in the intact closed-chest animal fail to provide evidence for participation of prostaglandins in regulation of coronary blood flow, normally or following brief periods of coronary artery occlusion. Afonso *et al.* (12), using closed-chest vagotomized dogs, also failed to find an effect of indomethacin on resting coronary blood flow. They did find however, that indomethacin slightly attenuates the response of the coronary vascular bed to hypoxia. In these experiments, indomethacin was given intravenously and the hypoxia was generalized.

Our data on the renal vascular effects of indomethacin are qualitatively in agreement with those studies showing an increase of renal vascular resistance (4–6). There is disagreement in the literature as to whether indomethacin impairs renal autoregulation. Herbaczynska-Cedro and Vane (4) reported that it does, but McNay and Miyazaki (6), in a preliminary communication, reported only a minor impairment. Our studies indicate that the renal vascular response to increasing perfusion pressure is unaffected, whereas the response to decreasing pressure is attenuated by direct intraarterial infusion of indomethacin. Herbaczynska-Cedro and Vane administered 1–5 mg/kg indomethacin intravenously by bolus injection and this increased mean arterial blood pressure by 5–20 mmHg. McNay and Miyazaki infused 10 mg/kg indomethacin intravenously. It is possible that systemic or local effects of indomethacin, independent of prostaglandin synthesis, contributed to the impairment of autoregulation in these studies. For example, it has been shown that raising renal vascular resistance by epinephrine infusion or cooling the blood also attenuates renal autoregulation (13). It is also possible that the impair-

ment of autoregulation was in fact related to inhibition of prostaglandin synthesis. However, the levels of prostaglandins may have been abnormally high in all three studies due, for example, to handling the kidney.

Indomethacin clearly attenuated renal reactive hyperemia. To our knowledge there are no comparable data in the literature. The possible mechanisms for attenuation of renal autoregulation described above could equally apply in this case. Thus the attenuation by indomethacin of renal reactive hyperemia may be related to, (a) a nonspecific effect, (b) inhibition of normal prostaglandin synthesis, and (c) inhibition of enhanced prostaglandin synthesis.

An interesting question posed by this and similar studies is: How does indomethacin cause an increase in renal vascular resistance? The correlation between renal output of a prostaglandin-like substance and renal blood flow (5) suggests that indomethacin, by blocking prostaglandin synthesis, abolishes the function of that hormone in maintaining renal blood flow. However, the rapidity with which indomethacin increases renal resistance on intra-arterial infusion suggests that other mechanisms may be involved. These could include direct effects of indomethacin on red cells, platelets, and vascular smooth muscle and indirect effects via the nervous system.

Summary. In two series of experiments we studied the effects of indomethacin on (a) coronary reactive hyperemia and, (b) renal blood flow, autoregulation, and reactive dilation. Coronary blood flow was measured in closed-chest dogs. Reactive hyperemia was induced by coronary occlusion for 5 and 15 sec. Indomethacin, an inhibitor of prostaglandin synthesis, was infused intra-arterially in doses of 90–200 mg over periods ranging from 30–120 min. Coronary reactive hyperemia was not affected by indomethacin. The canine renal vascular bed was studied under conditions of natural flow, controlled flow, and controlled pressure. Intra-arterial infusion of 90 mg of indomethacin over a 30- to 60-min period caused increased renal vascular resistance and an attenuation of reactive dilation (induced by stopping renal blood flow for 90 sec). Indomethacin slightly attenuated the autoregulatory response to decreasing perfusion pressures, but did not affect the response to increasing pressures. Thus the study fails to provide evidence for participation of the prostaglandins in regulation of coronary blood flow and suggests only minimal participation of prostaglandings in renal blood flow regulation.

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