CONSIDERABLE evidence exists which implicates prostaglandins as important mediators of renal renin secretion. Central to this hypothesis is the observation that prostaglandin cyclooxygenase inhibitors prevent the renin secretion induced by several stimuli, including intrarenal baroreceptor activation (Data et al., 1979; Berl et al., 1979; Henrich et al., 1979), macula densa activation (Henrich et al., 1979; Olson et al., 1980), blockade of the angiotensin II short-loop negative feedback mechanism (Campbell et al., 1979a; Abe et al., 1980), and, in some species, β-adrenoreceptor stimulation (Campbell et al., 1979b; Feuerstein and Feuerstein, 1980). Since the renin-angiotensin system may be responsible for the elevated arterial blood pressure in certain types of experimental and clinical hypertension, inhibition of renin release by cyclooxygenase inhibitors represents a potential antihypertensive action of this class of drugs. Hypothetically then, in renin-dependent hypertension in which the hyperreninemia is prostaglandin mediated, cyclooxygenase inhibitors should alleviate both the hyperreninemia and hypertension.

Total ligation of the aorta between the origin of the renal arteries in rats produces severe renovascular hypertension which is renin dependent (Carretero et al., 1971; Sweet et al., 1979; Ferrandes et
al., 1978; Sweet and Columbo, 1979). Further, since most forms of renin release in the rat examined thus far are prostaglandin mediated (Leyssac et al., 1978; Campbell et al., 1979a, 1979b), it seemed likely that the hyperreninemia resulting from aortic ligation is also dependent upon renal prostaglandin synthesis. Therefore, the current study was designed to (1) determine if the excessive renin secretion in aortic ligated rats is prostaglandin mediated, and (2) observe the effects of cyclooxygenase inhibition on arterial blood pressure in a model of hypertension which is secondary to severe hyperreninemia.

**Methods**

Male Sprague-Dawley rats (250-300 g) obtained from Harlan Labs were used in all experiments. Rats were maintained on a diet of Wayne Lab-Blox (Allied Mills, Inc.) containing 170 mEq Na+/kg and 246 mEq K+/kg and tap water ad libitum.

Under ether anesthesia, complete ligation of the aorta between the origin of the renal arteries was done (No. 0000 silk thread), taking meticulous care to avoid occlusion of the left renal artery and mesenteric artery (Rojo-Ortega and Genest, 1968). In some rats, a left nephrectomy was performed at the time of aortic ligation. Six days after aortic ligation, the animals were lightly anesthetized with ether and a chronic carotid cannula (Silastic, 0.02" i.d. x 0.037" o.d.) was implanted and exteriorized to the dorsal neck region. The study was begun on day 6 because arterial blood pressure rises rapidly after aortic ligation and does not plateau until that time (Sweet et al., 1976).

Four to 6 hours after the second surgical manipulation, the carotid cannula was connected to a Statham pressure transducer (Statham Instruments) and blood pressure was recorded on a Hewlett Packard physiograph (model 7758A). All measurements were obtained in the conscious unrestrained rat. Each rat was allowed an interval of 30 minutes to acclimatize to its surroundings before the measurements, approximately 750 μl of blood were drawn from the carotid artery into heparin (5 U/ml final concentration). The blood was immediately centrifuged at 4°C and the plasma separated and stored at -20°C until assayed for plasma renin activity (PRA).

Rats were injected with either olive oil (1 ml/kg, sc) or indomethacin (Sigma, 5 mg/kg, sc) suspended in olive oil at 5:00 p.m. and again at 8:00 a.m. and 12:00 p.m. the following day (total dose of 15 mg/kg per 24 hours). This dosage regimen was selected on the basis of previous studies that demonstrated that indomethacin, 5 mg/kg, administered subcutaneously to rats inhibits renal prostaglandin E2 production by 89% over an 8-hour period (Campbell et al., 1979b). Two to 5 hours after the last dose of indomethacin, MABP and HR were again determined and samples of blood were obtained for PRA determinations.

Plasma renin activity was determined by an angiotensin I radioimmunoassay kit (Squibb) and the results were expressed as nanograms of angiotensin I generated at 37°C per milliliter of plasma per hour (ng AI/ml per hr). Statistical analyses were performed with either a two-tailed paired or unpaired Student's t-test. All values are expressed as mean ± SEM.

**Results**

Preliminary studies were conducted to determine the reproducibility of aortic ligations-induced hyperreninemic hypertension. In 16 aortic ligated rats in which the left ischemic kidney had been removed at the time of coarctation, MABP and PRA were 130.1 ± 3.4 mm Hg and 0.28 ± 0.09 ng AI/ml per hr, respectively, 6 days after aortic ligation (Fig. 1). In 26 other rats, in which the ischemic kidney was left intact, aortic ligation was associated with a significantly higher MABP of 180.7 ± 4.0 mm Hg (P < 0.001) (Fig. 1). PRA was determined in 24 of these animals (blood samples unobtainable in two) and was significantly elevated (14.9 ± 3.4 ng AI/ml per hr) when compared to aortic ligated plus left nephrectomized animals (P < 0.001). These values for MABP and PRA 6 days after aortic ligation are in close agreement with previously reported values (Sweet et al., 1976).

Figure 2 illustrates the effects of indomethacin treatment on PRA and MABP in another group of aortic ligated rats. After indomethacin treatment, PRA decreased by 56% from 21.2 ± 7.3 ng AI/ml per hr to 9.3 ± 2.9 ng AI/ml per hr (P < 0.045, n
Concomitant with this decrease in PRA there was a 13% reduction in MABP from 179.0 ± 6.7 to 156.4 ± 8.8 mm Hg (P < 0.002, n = 11). The percentage decrease in PRA and decrease in MABP following indomethacin treatment were significantly correlated (r = 0.766, P < 0.016) (Fig. 3). Unlike PRA and MABP, HR was not affected by indomethacin treatment (431 ± 18 beats/min vs. 410 ± 15 beats/min before and after indomethacin, respectively).

A control group of aortic ligated rats was treated with vehicle without indomethacin (olive oil, 1 ml/kg, sc) in a fashion similar to the group of rats that received indomethacin. In these rats, PRA and MABP were not significantly affected, although PRA did tend to increase somewhat during the vehicle treatment (Fig. 4).

The toxic effects of indomethacin on the gastrointestinal tract of rats have been well documented (Fang et al., 1977; Soldato and Meli, 1978; Brown et al., 1978; Kauffman et al., 1979; Cioli et al., 1979). In our studies, autopsies of rats treated with indomethacin for 21-24 hours revealed some non-perforating gastric ulcerations, but not macroscopic intestinal lesions or peritonitis. Nevertheless, the possibility existed that the hypotensive re-
response to indomethacin in aortic ligated rats was due to the toxic effects of indomethacin, independent of its effects on renin secretion. To evaluate this possibility, the effects of indomethacin on MABP in rats with both aortic ligation and left nephrectomy were examined (Fig. 5). In these low renin animals, the MABP was not affected by indomethacin.

Discussion

In the aortic ligation model of hypertension in the rat, indomethacin induces an unequivocal fall in blood pressure at the same time it inhibits renin secretion. The blood pressure response to indomethacin in this model contrasts to reports of increases in pressure observed in alternative models of hypertension (Romero et al., 1975; Pugsley et al., 1975a, 1975b; Strong and Romero, 1976; Romero and Strong, 1977; Cangiano et al., 1978). However, a feature of these models is that the contribution of the renin-angiotensin system to the blood pressure elevation is equivocal. These latter studies have led to the suggestion that cyclooxygenase inhibitors reduce the production of vasodilatory and natriuretic prostaglandins, and therefore accentuate the hypertension. In other studies, indomethacin either has not influenced blood pressure (Zimmerman 1978; Ylitalo et al., 1978; Yun et al., 1979) or, in high plasma renin activity, has lowered blood pressure. In particular, indomethacin lowered the blood pressure in a patient with bilateral renal artery stenosis (Frohlich et al., 1979), in two siblings with renin-dependent hypertension, hyperaldosteronism and hypokalemia (DeJong et al., 1980), and in conscious sodium-depleted dogs (DeForrest et al., 1980). It is well recognized that the prostaglandin system has an integral role in the mechanisms of renin release (Oates et al., 1979); thus, it is possible that cyclooxygenase inhibition of prostaglandin production could result in decreases in blood pressure in renin-dependent hypertension, in which the hyperreninemia is prostaglandin mediated. Evaluation of this hypothesis would be contingent on acquiring a model of renin-dependent hypertension, in which the excessive renin secretion is prostaglandin mediated.

Ligation of the aorta between the renal arteries of rats results in an ischemic left kidney and induces a severe form of hypertension associated with high plasma renin activities (Fig. 1). The mechanism for the elevation of renin levels in this model and the possible relationship of this excessive renin secretion to the prostaglandin system has not previously been evaluated. Assuming that the indomethacin-induced response on plasma renin activity was due to inhibition of prostaglandin synthesis, the reduction in hyperreninemia (Fig. 2) provides evidence that prostaglandins mediate the excessive renin secretion in this model. This would be consistent with the involvement of prostaglandins in mediating many stimuli for renin secretion in this species (Leyssac et al., 1975; Campbell et al., 1979a, 1979b).

Once elevated, the high level of renin activity is considered to contribute in a major way to the development of hypertension in this specific model of hypertension. This conclusion is based on the observations that plasma renin activity is elevated at least 10-fold in aortic ligated rats (Carretero et al., 1971; Sweet et al., 1976, Sweet and Columbo, 1979; and Figure 1) and that removal or inactivation of the renin-angiotensin system by nephrectomy (Carretero et al., 1971; Fernandes et al., 1978; Sweet and Columbo, 1979; and Figure 1), anti-angiotensin II antibodies (Carretero et al., 1971), angiotensin antagonists (Sweet et al., 1976; Fernandes et al., 1978; Sweet and Columbo, 1979), or converting-enzyme inhibitors (Sweet and Columbo, 1979) abolishes the hypertension. In this renin-dependent model of hypertension, indomethacin treatment lowered mean arterial blood pressure (Fig. 2). This was unlikely to be a non-specific effect due to indomethacin toxicity, as similar indomethacin administration to aortic ligated plus left nephrectomized animals failed to reduce blood pressure (Fig. 5). Although this low renin control group was not as hypertensive as the aortic ligated rats with an intact left ischemic kidney, these experiments indicate that the hypotensive action of indomethacin was not due to gastrointestinal toxicity, peritonitis, and shock. Along these lines, it is relevant to mention that we have examined the effects of indomethacin on the mean arterial blood pressure of aortic ligated plus left nephrectomized spontaneously hypertensive rats. These animals were hypertensive due to a genetic mechanism, had low plasma renin activities due to removal of the left ischemic kidney, and had been subjected to the surgical stress of aortic ligation. In these low renin, hypertensive, aortic ligated animals, 24-hour indomethacin treatment increased, rather than decreased, mean arterial blood pressure in each of the three rats investigated (173 ± 12 mm Hg vs. 198 ± 12 mm Hg). Since plasma renin activity was high in the aortic ligated rats and low in the aortic ligated plus left nephrectomized spontaneously hypertensive rats, it appears that the hypotensive action of indomethacin was dependent upon the presence of excessive renin secretion prior to treatment. Further support for a cause and effect relationship was a significant correlation between the decrease in mean arterial blood pressure and the percentage decrease in plasma renin activity following indomethacin treatment in aortic ligated rats (Fig. 3). This correlation indicated that at least 59% of the variability in mean arterial blood pressure following indomethacin administration was explainable on the basis of changes in plasma renin activity. Since the correlation coefficient for this association was only 0.766, physiological changes besides alterations in plasma renin activity may have contributed to the hypotensive action of indomethacin. However,
the observed r value probably reflects the analytical variability associated with plasma renin activity determinations and the inherent variability of blood pressure measurements in conscious animals, rather than the result of other hypotensive mechanisms. Although a significant correlation does not prove a direct relationship, the renin-dependent nature of this model strongly suggests that indomethacin lowers blood pressure in aortic ligated rats by inhibiting prostaglandin-mediated renin release.

If the hypothesis that excessive renal prostaglandin production can result in renin-dependent hypertension is true, then chronic infusions of renin-releasing prostaglandins into the renal artery of an experimental animal should mimic renovascular hypertension. Support for this hypothesis has been reported recently by Hockel and Cowley (1979) who demonstrated that chronic intrarenal prostaglandin E₂ infusions produced both elevated plasma renin activity and hypertension in dogs. Further, the rise in blood pressure during intrarenal prostaglandin E₂ infusions had an almost perfect linear relationship with the rise in plasma renin activity.

As mentioned previously, with other experimental models, prostaglandin synthesis inhibition may exacerbate hypertension. Romero and Strong (1977) have clearly demonstrated that chronic indomethacin treatment increases the blood pressure of one-kidney Goldblatt rabbits. Furthermore, indomethacin induced malignant hypertension in those two-kidney Goldblatt rabbits in which renal artery constriction was associated with a decrease in renal blood flow. Similarly, Colina-Chouria and co-workers (1979) reported that chronic indomethacin administration to normotensive rabbits also increased arterial blood pressure. Several explanations are possible for these apparent discrepancies. First, species differences must be taken into account. In the rat kidney, prostaglandin E₂ is considered to be a vasoconstrictor whereas, in the rabbit kidney, prostaglandin E₂ is a vasodilator (Malik and McGiff, 1975). Second, the time frame of indomethacin administration may be considered. The effects of indomethacin on blood pressure in rabbits were assessed over several days, whereas the present study was necessarily limited to 24 hours because of the gastrointestinal toxicity of chronic indomethacin treatment in rats. Third, the qualitatively different response to indomethacin observed in aortic ligated rats may relate to the high degree of renin-dependency of this hypertensive model. Prostaglandin synthesis inhibitors prevent the synthesis of vasodilatory and natriuretic prostaglandins and inhibit the release of renin. The former effect is potentially hypertensive, whereas the latter effect is potentially antihypertensive. The net effect, then, of prostaglandin synthesis inhibition on blood pressure would depend upon which is more prominent, prostaglandin-mediated vasodilation or prostaglandin-mediated renin release. This, of course, would depend upon the experimental model under investigation. Since the blood pressure elevation in aortic ligated rats is unequivocally renin dependent, it is not surprising that indomethacin lowers blood pressure in this hypertensive model.

In summary, aortic ligation in rats produces severe renovascular hypertension which is, in part, secondary to prostaglandin-mediated renin release. In addition, these studies demonstrate that, in this model of renin-dependent hypertension, inhibition of prostaglandin synthesis with indomethacin can alleviate, rather than exacerbate, the hypertension.

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