Effect of β-adrenergic blockade and inhibitors of angiotensin II and prostaglandins on renal autoregulation

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The mechanism of renal autoregulation remains the center of considerable controversy. The effect of papaverine to abolish renal autoregulation has been used to support the myogenic theory of renal autoregulation in which transmural tension across the afferent arteriole is proposed as the determinant of the relative state of vascular tone (37). Alternatively, smooth muscle paralysis with papaverine could render the afferent arteriole unresponsive to any intrarenal mechanism most frequently proposed to be involved in renal autoregulation. In view of the presence of inhibition of prostaglandin synthetase with indomethacin and meclofenamate in the in situ perfused dog kidney, there is no ready explanation why a high-sodium intake and mineralocorticoid administration have been reported to blunt renal autoregulation in the isolated perfused dog kidney (18) and in situ perfused kidney (6). Other circumstantial evidence against the renin-angiotensin system as the regulator of renal autoregulation is the finding that renal venous (11, 26) and lymph angiotensin II concentrations (3) increase as renal vasodilatation occurs. A decrease in renin activity, and thus the vasoconstrictor effect of angiotensin II, might be expected to be associated with renal vasodilatation should a cause-and-effect relationship exist.

More recently another intrarenal hormone has been suggested to be involved in renal autoregulation. Inhibition of prostaglandin synthetase in the kidney with the administration of indomethacin or meclofenamate has been found to decrease inner cortical and presumably medullary blood flow (17, 19, 25). More recently indomethacin has been reported to nearly completely abolish autoregulation of blood flow in the in situ perfused kidney (15) and the renal vasodilatory response to hemorrhagic hypotension (39). Since angiotensin II has been shown to increase the renal release of angiotensin II (22). Renal autoregulation was also examined in animals pretreated with a competitive antagonist of angiotensin II, [1-sarcosine, 8-glycine] angiotensin II, or one of two chemically dissimilar inhibitors of prostaglandin synthetase, indomethacin and meclofenamate. Because of recent evidence suggesting a role for an intrarenal beta receptor in regulating renin release, renal autoregulation was also examined in animals treated with the beta-adrenergic blocking agent propranolol. In all groups of animals constancy of glomerular filtration rate (GFR) and renal blood flow (RBF) was observed after substantial decreases in RAP to a range of 70-90 mmHg. These studies therefore do not provide evidence in support of a role for angiotensin II, prostaglandins, or an intrarenal beta receptor as mediators of the renal autoregulation of GFR or total RBF.

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tized with intravenous pentobarbital (30 mg/kg), then intubated and ventilated with a Harvard respirator (Harvard Apparatus Co. Inc., Millis, Mass.). Supplemental doses of pentobarbital were administered as needed throughout the experiment to maintain a stable state of anesthesia. Polyethylene catheters were placed in both ureters and renal veins through bilateral flank incisions made by retroperitoneal approach. A Blalock clamp was placed around the aorta above both renal arteries to allow alteration in renal arterial pressure (RAP). Catheters were placed in the brachial and femoral arteries to obtain blood samples and to allow monitoring of arterial blood pressure above and below the aortic clamp with Statham transducers (Statham Instruments Inc., Oxnard, Calif.). A 0.9% sodium chloride solution containing sufficient inulin and $p$-aminohippurate (PAH) was infused (0.5 ml/min) into a forearm vein to maintain plasma levels of these substances at 15-20 mg/100 ml and 1-3 mg/100 ml, respectively. To allow replacement of fluid losses and adequate urine flows for accurate clearance measurements 2.5% dextrose was infused intravenously at 10 ml/min for 50 min during surgery. Thirty minutes after completion of surgery an additional 600 ml of 2.5% dextrose were administered at 10 ml/min and then the infusion rate was adjusted to equal urine flow rate.

In the propranolol (group I), indomethacin (group III), and meclofenamate (group IV) studies three urine collections 5-10 min in duration were made before and after administration of these agents. The postdrug urine collections were made after a 20-30 min equilibration period after drug administration and served as the control periods for the periods during the first lowering of RAP by aortic constriction. In the angiotensin II inhibitor studies (group II) the pressor response to 500 ng of angiotensin II was examined before and after administration of the inhibitor. Three control urine collections then were obtained prior to the aortic constriction periods.

In all studies RAP was lowered in 20- to 25 mmHg decrements. After each decrement an equilibration period of 15-30 min was allowed before blood and urine samples were collected. Arterial and renal venous samples were obtained at the midpoint of alternate urine collections. In all studies two or three sequential aortic clamping periods were obtained in each animal prior to the aortic clamp release. The urine collections during the first clamping period were complete for groups I-IV within 1 h after drug administration. All sequential clamping periods were complete for groups I-IV within 2 h of drug administration.

In some indomethacin studies (group III) renal blood flow was determined by a square-wave electromagnetic flow probe placed around a renal artery (Carolina Medical Electronics, King, N.C.). In these studies a zero base line was obtained prior to the studies by temporary occlusion of the renal artery.

**Group I—propranolol studies.** In these studies the effect of beta-adrenergic blockade on renal autoregulation was examined when propranolol, 2 mg/kg (4 dogs) or 4 mg/kg (2 dogs), was administered by intravenous injection and then propranolol, 0.4 mg/kg per h, in 0.9% sodium chloride solution was infused intravenously at 0.5 ml/min throughout the experiment. After 30 min and stabilization of urine flow three to five control periods were obtained; RAP then was decreased 20-25 mmHg by adjustment of the supraprenal aortic clamp. Experimental collections for three to five periods were commenced after a 20- to 30-min equilibration period at the lower RAP. In most animals a second, and occasionally a third, lowering of RAP by another 20-30 mmHg was accomplished and after another equilibration experimental collections again were made. Urine collections were also made after release of the aortic clamp. During the control period and with each alteration in RAP plasma renin activity (PRA) was measured by the radiimmunoassay technique of Stockigt, Collins, and Biglirici (32).

**Group II—angiotensin inhibitor studies.** In five dogs renal autoregulation was examined during the infusion of [1-sar-cosine, $p$-glycinelangiotensin II, a substance known to be a competitive inhibitor of angiotensin II (22) and obtained from Dr. D. Regoli, Sherbrooke, Quebec. This angiotensin inhibitor was infused at 0.5 ml/min iv (4 dogs) or into the aorta just above orifices of the renal arteries (1 dog) at a rate of 1 $\mu$g/kg per min in a 0.9% sodium chloride solution. The same protocol described in the group I studies to examine the effects of increasing and decreasing RAP was also used in these group II studies.

**Group III—indomethacin studies.** In eight dogs renal autoregulation was examined after inhibition of prostaglandin synthesis with a single intravenous injection of indomethacin in a dose of either 2 mg/kg (6 dogs) or 10 mg/kg (2 dogs). The same protocol described for the group I studies was used in these experiments. In five dogs a 2-mg/kg dose of indomethacin was injected intravenously at the time of each reduction in renal perfusion pressure. Urine and blood collections were then started within 15 min of indomethacin administration. In an additional five dogs, renal autoregulation was examined with an electromagnetic flow probe after either 10-mg/kg (2 dogs) or 20-mg/kg (3 dogs) doses of indomethacin.

**Group IV—meclomenamate studies.** Renal autoregulation was examined in these studies (4 dogs) in the presence of inhibition of prostaglandin synthesis with meclofenamate in a 2-mg/kg intravenous bolus followed by a constant infusion of 2 mg/kg per h in 0.9% sodium chloride solution at a rate of 0.5 ml/min. Otherwise the same protocol described for the group I studies was used in these experiments.

The calculations used in the present experiments have been discussed elsewhere (30). $p$-Aminohippurate was determined by the method of Bratton and Marshall as adapted for the Technicon AutoAnalyzer (14). Inulin was determined colorimetrically by reaction with antthone in H$_2$SO$_4$ (8) by a technique adapted for the Technicon AutoAnalyzer (9). The analysis of variance was used when several values were involved and the paired Student $t$ test when two values were involved. A $P$ value of $<0.05$ was considered significant. All data are expressed as means $\pm$ SE.

**RESULTS**

**Effect of propranolol on renal autoregulation** (Fig. 1). The results for the studies in group I were similar for both doses of propranolol used; the results therefore were analyzed together. In these studies control measurements of renal hemodynamics were made before and after the administration of propranolol. There was no significant difference in
mean arterial pressure (MAP) before (132 ± 5 mmHg) and after (127 ± 5 mmHg) administration of propranolol. However, three animals did become profoundly hypotensive after propranolol administration and therefore were not used for the study. Glomerular filtration rate and RBF also were not altered by beta-adrenergic blockade with intravenous propranolol; GFR was 49 ± 4 before and 50 ± 5 ml/min after propranolol and RBF was 215 ± 20 and 207 ± 23 ml/min, respectively. In addition, renal vascular resistance (RVR) did not significantly change after propranolol (0.656 ± 0.062 before and 0.645 ± 0.056 mmHg/ml per min after propranolol). The results in the group I experiments for serial decrements in RAP on GFR and RBF are shown in Fig. 1. The constancy of these parameters is apparent. No significant differences in GFR or RBF occurred at any level of RAP. Control values for RAP (127 ± 5 mmHg) were not significantly different from values obtained after release of the aortic clamp at the end of the experiment (128 ± 7 mmHg). Values for GFR (50 ± 5 to 49 ± 3 ml/min), RBF (207 ± 23 to 220 ± 16 ml/min), and RVR (0.645 ± 0.060 to 0.640 ± 0.07 mmHg/ml per min) also were not significantly different from control values after release of the aortic clamp. The mean RVR for all periods both before clamping and after clamp release was 0.642 ± 0.060 mmHg/ml per min. This value was significantly higher than the RVR of 0.416 ± 0.037 mmHg/ml per min (P < 0.001) that was present when RAP was decreased to 79 ± 1 mmHg by aortic clamping. After propranolol administration PRA decreased from 32.4 ± 8 to 10.6 ± 3 ng/ml per h (P < 0.02). A decrease in RAP from 127 ± 5 to 104 ± 3 mmHg was associated with an increase in PRA from 10.6 ± 3 to 15.9 ± 4 ng/ml per h (P < 0.1) and a further decrease in RAP to 79 ± 1 mmHg increased PRA to 23.7 ± 5 ng/ml per h (P < 0.05). Release of the clamp was associated with a decrease in PRA to 10.4 ± 3 ng/ml per h, a value significantly below (P < 0.005) the PRA of 23.7 ± 5 ng/ml per h prior to clamp release.

**Effect of angiotensin II inhibitor on renal autoregulation (Fig. 2).**

Prior to infusion of the inhibitor, 500 ng of angiotensin II increased blood pressure by 27 ± 4 mmHg; the same dose of angiotensin II did not alter blood pressure after infusion of the inhibitor. The effect of the angiotensin inhibitor on the renal vascular response to 500 ng of angiotensin II also was tested in eight experiments performed on four kidneys. In these experiments, 500 ng of intravenous angiotensin II resulted in a significant decrease in RBF as determined by electromagnetic flow probe (253 ± 28 to 209 ± 29 ml/min, P < 0.001). However, in the same animals undergoing an intravenous infusion of 1 μg/kg per min of [L-sarcosine, 8-glycine]angiotensin II, the same dose of angiotensin II did not affect RBF (243 ± 25 to 242 ± 24 ml/min). The results in the group II experiments for serial decrements in RAP on GFR and RBF are shown in Fig. 2. No significant changes occurred in GFR or RBF at any level of RAP. There was no significant change in GFR (51 ± 2 to 51 ± 2 ml/min) or MAP (149 ± 3 to 149 ± 3 mmHg) when values before aortic clamping and after aortic clamp release were compared. There was, however, a significant increase in RBF from 250 ± 21 to 336 ± 18 ml/min (P < 0.01) when preclamp and clamp-release values were compared. We have no ready explanation for this increase. The mean RVR for all periods both before aortic clamping and after clamp release was 0.456 ± 0.030 mmHg/ml per min and, as RAP was decreased to 88 ± 3 mmHg, RVR fell significantly to 0.274 ± 0.026 mmHg/ml per min (P < 0.001). The RBF generally was higher in the group treated with angiotensin inhibitor than in other groups because all animals except one were greyhounds with high hematocrits.
Effect of indomethacin on renal autoregulation (Fig. 3). The intravenous injection of 2 mg of indomethacin/kg resulted in increases in MAP (155 ± 4 to 165 ± 5 mmHg), GFR (51 ± 6 to 53 ± 5 ml/min), and RVR (0.804 ± 0.064 to 0.916 ± 0.069 mmHg/ml per min) whereas RBF decreased (207 ± 19 to 189 ± 13 ml/min). These values, however, were not significantly different. The administration of 10 mg/kg of indomethacin did not significantly alter MAP (154 ± 8 to 156 ± 6 mmHg) or GFR (41 ± 4 to 43 ± 4 ml/min). However, this dose of indomethacin significantly lowered RBF (236 ± 12 to 103 ± 11 ml/min, P < 0.02) and increased RVR (0.652 ± 0.011 to 0.872 ± 0.081 mmHg/ml per min, P < 0.05). Serial decrements in RAP produced little change in GFR or RBF in animals pretreated with either 2 or 10 mg of indomethacin/kg (Fig. 3). The results for the 2- and 10-mg/kg doses were similar; the data therefore were averaged together. No significant changes in GFR or RBF at any level of RAP were present. There was no significant difference in MAP (161 ± 4 to 153 ± 6 mmHg), GFR (51 ± 4 ml/min), RBF (187 ± 10 to 215 ± 23 ml/min), or RVR (0.904 ± 0.054 to 0.082 ± 0.098 mmHg/ml per min) when preclamp and clamp-release values are compared. The mean RVR for all periods both before clamping and after clamp release was 0.569 ± 0.055 mmHg/ml per min (P < 0.001) that occurred at a mean RAP of 86 ± 3 mmHg. In all these studies the periods during the first aortic clamping were performed within 1 h of indomethacin administration and all clampings were performed within 2 h of indomethacin administration. However, to ensure maximal action of the drug five dogs were studied immediately after indomethacin administration, 2 mg/kg. In these animals, renal perfusion pressure was decreased from 140 ± 8 to 85 ± 4 mmHg, neither GFR (41 ± 3 to 39 ± 4 ml/min) nor RBF (181 ± 17 to 156 ± 15 ml/min) was significantly altered.

Additional experiments were performed in five animals treated with either a 10- or 20-mg/kg intravenous bolus of indomethacin in which RBF was measured by electromagnetic flow probe. The results were the same with both dosages and thus the data are analyzed together. Immediately after indomethacin, a significant increase in MAP (145 ± 10 to 153 ± 10 mmHg, P < 0.05) was noted. Immediately after indomethacin, a significant fall in RBF was also observed (266 ± 46 to 205 ± 44 ml/min, P < 0.01). In these studies, decreasing RAP from 153 ± 6 to 84 ± 7 mmHg did not result in significant alterations in RBF (205 ± 41 to 200 ± 24 ml/min) but a significant decrease in RVR (0.575 ± 0.09 to 0.406 ± 0.06 mmHg/ml per min, P < 0.025) was observed. Upon return of RAP to control values (146 ± 5 mmHg) both RBF (212 ± 25 ml/min) and RVR (0.684 ± 0.10 mmHg/ml per min) were at precontrol levels.

Effect of meclofenamate on renal autoregulation (Fig. 4). In these studies, 2 mg/kg of meclofenamate resulted in a significant increment in MAP (146 ± 6 to 160 ± 8 mmHg, P < 0.05) and RVR (0.600 ± 0.072 to 0.789 ± 0.096 mmHg/ml per min, P < 0.05) as RBF decreased (227 ± 17 to 180 ± 18 ml/min, P < 0.001) in the three animals in which the measurements were made. Serial decrements in RAP produced little change in GFR or RBF in the meclofenamate-treated animals (Fig. 4). No significant differences in GFR or RBF at any level of RAP were present. There were no significant changes in GFR (49 ± 3 to 46 ± 5 ml/min), RBF (185 ± 14 to 191 ± 21 ml/min), or RVR (0.769 ± 0.096 to 0.678 ± 0.113 mmHg/ml per min) when pre- and postaortic clamp values were compared, even though MAP decreased significantly from 160 ± 8 to 134 ± 5 mmHg (P < 0.02). With a decrease in RAP from control values to 76 ± 1 mmHg, RVR was significantly decreased from the mean value during preclamp and clamp-release periods of 0.727 ± 0.074 to 0.464 ± 0.038 mmHg/ml per min (P < 0.005).

DISCUSSION

In the present study several methods were used to investigate the potential role of humoral mediators in the autoregulation of GFR and RBF. In one group of studies beta-
adrenergic blockade with propranolol was used to lower control levels of PRA. This effect of propranolol was not found to impair renal autoregulation of either GFR or RBF. In these studies, despite beta-adrenergic blockade with propranolol, graded aortic clamping was found to sequentially increase PRA as RVR decreased. Thus, as in previous studies (11, 26) an inverse, rather than a direct, relationship was found between RVR and PRA. These results also confirm the findings of other investigators that renin release may occur as RVR decreases (10) and thus as the stress on any intrarenal baroreceptor controlling renin release presumably increases (5). These findings are therefore in contrast to the original proposal that a fall in renal perfusion pressure increases renin release by decreasing stretch on an intrarenal vascular receptor (31), since the renal vasodilatation would suggest increased stretch on a baroreceptor located at the site of afferent resistance. Although propranolol did not block increases in PRA with graded decrements in RAP in the present study, we have recently demonstrated that this dose of propranolol does result in blockade of renal renin release in response to stimulation of intrarenal beta adrenergic receptors (33). Hence, although the propranolol studies do not exclude an intrarenal role for renin in renal autoregulation, they do exclude a role for an intrarenal beta-adrenergic receptor. Finally, the significant fall in PRA with propranolol in the control period in these anesthetized animals without significant changes in GFR, RBF, or RVR would suggest that some of the basal renin level is due to stimulation of beta-adrenergic receptors.

Another approach was also used in the present study to implicate the renin-angiotensin system in renal autoregulation. Renal autoregulation was examined during the infusion of a competitive inhibitor of angiotensin II, [1-sarcosine, 8-glycine]angiotensin II. The dose of this inhibitor used in the present study was shown to completely abolish the pressor response and the renal vasoconstrictor response to an intravenous injection of 500 ng of angiotensin II. We have also recently found that even smaller doses of this inhibitor block the effect of angiotensin II to suppress renin release (22). In the present study, the continuous administration of this effective inhibitor of the vascular effects of angiotensin II failed to affect the autoregulation of either GFR or RBF. These findings are compatible with recent preliminary communications from other laboratories that demonstrate that neither the intrarenal infusion of an inhibitor of angiotensin-converting enzyme (15) nor another competitive inhibitor of angiotensin II ([1-sarcosine, 8-alanine]angiotensin II) (27) diminishes the autoregulation of RBF in response to decreases in renal perfusion pressure. Taken together, these results fail to provide support for the hypothesis that renal autoregulation is dependent on the integrity of the renin-angiotensin system.

The present investigation also examined the role of prostaglandins in renal autoregulation of GFR and RBF. In a recent study, Vatner (39) found that indomethacin reduced the renal vasodilatory response to hemorrhagic hypotension. However, in these animals, decreases in cardiac output and blood volume, in addition to decrements in renal perfusion pressure, were present. The results of another recent study by Herbaczynska-Cedro and Vane (15) have suggested that inhibition of prostaglandin synthetase with indomethacin is associated with impairment of renal autoregulation of total RBF. These authors, however, performed their studies in cannulated perfused kidneys in which the level of RVR was abnormally high. In these studies the administration of indomethacin to inhibit prostaglandin synthesis was associated with a further rise in RVR to an even higher level (15). Although the absence of autoregulation of RBF in high-resistance, perfused kidneys is of interest, it seemed important to examine the effect of inhibition of prostaglandin synthetase on renal autoregulation in noncannulated, nonperfused kidneys that have lower levels of RVR. In the present investigation such studies were performed and autoregulation of RBF and GFR was found not to be impaired by inhibition of prostaglandin synthetase.

One question that could be raised about our results is whether renal prostaglandin inhibition was achieved at the time renal autoregulation was tested. In this regard, it is important to emphasize that the doses of indomethacin and meclofenamate used in the present study are greater than doses that have been shown to significantly diminish the release of prostaglandins into renal venous blood (2, 15) and to abolish the conversion of labeled arachidonic acid to prostaglandin in renal medullary tissue (21). In addition, these doses of indomethacin and meclofenamate are equivalent to or greater than those used by Herbaczynska-Cedro and Vane (15) in a perfused kidney system in which renal autoregulation was reported to be abolished. Moreover, previous studies have demonstrated that angiotensin-stimulated release of renal prostaglandin is inhibited longer than 1 h after a 1 mg/kg iv dose of meclofenamate and is depressed by 50% at 3 h after a single 1- or 2-mg/kg iv dose of indomethacin (2). Finally, using a specific radioimmunoassay, recent investigators have demonstrated that a single 2-mg/kg iv dose of indomethacin significantly depresses prostaglandin E1 in renal venous blood for more than 2.5 h (40).

In the present study the preservation of autoregulation of GFR and RBF was demonstrated at 1 and 9 h after treatment with indomethacin (2-20 mg/kg). Also studies were performed in which RAP was lowered immediately after indomethacin administration and renal autoregulation was similarly present in these studies. Large doses of meclofenamate (2 mg/kg plus 2 mg/kg per h) also failed to alter autoregulation of GFR and RBF in the present studies. On the basis of previous studies (2, 15, 21, 40) there can be little doubt that autoregulation was tested in the present study in the presence of substantial inhibition of prostaglandin synthesis.

The present studies therefore indicate that renal autoregulation of GFR and total RBF is not altered in the in situ kidney by rather large doses of two chemically dissimilar inhibitors of prostaglandin synthetase. These results are compatible with the findings reported in three recent preliminary communications in which no significant alteration in autoregulation of RBF occurred in the presence of inhibition of prostaglandin synthesis (1, 24, 25). Although prostaglandin release may not be a significant determinant of autoregulation of total RBF, some disturbance of autoregulation of medullary blood flow may occur after prostaglandin inhibition (24). Also in this regard, inhibitors of prostaglandin synthesis have been shown to decrease inner cortical blood flow (17, 19, 24). Finally, there is also some evidence that
renal prostaglandins may mediate the reactive hyperemia that occurs after renal arterial occlusion (16, 25).

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REFERENCES