Role of Medullary Hemodynamics in the Natriuresis of Drug-Induced Renal Vasodilation in the Rat

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With the technical assistance of M.A. WATERLOOS and V. VANDERBIESEN

SUMMARY In contrast to most renal vasodilators, such as acetylcholine (ACh), secretin increases renal blood flow in the dog without a marked effect on sodium excretion (U\text{Na}\text{V}). To investigate this observation, we studied the relationship between renal vasodilation, U\text{Na}\text{V}, and papillary plasma flow (PPF) in rats, infused either with ACh or secretin into the aorta at the level of both renal arteries. ACh significantly increased GFR, PAH clearance, and U\text{Na}\text{V} (A + 0.35 ml/min, + 2.11 ml/min and + 1.77 μEq/min, respectively; \(P < 0.05\)). PPF rose from 50 ± 2.6 ml/min 100 g (mean ± SE) in control rats to 91 ± 4.7 ml/min 100 g after ACh (\(P < 0.001\)). Despite a similar increase in PAH clearance after secretin (+ 2.21 ml/min; \(P < 0.01\)), U\text{Na}\text{V} remained unchanged and PPF was only slightly, although significantly, increased (from 50 ± 2.6 ml/min 100 g to 65 ± 2.75 ml/min 100 g; \(P < 0.05\)). Both total kidney and papillary vasodilation, and the increase in U\text{Na}\text{V} after ACh were blocked by previous administration of meclofenamate (M), a prostaglandin inhibitor. No effect of M in secretin-infused rats was observed. In conclusion, the relationship between total renal vasodilation and natriuresis was dissociated with secretin, but not with ACh. However, a relationship between the natriuresis and influence on papillary hemodynamics was observed with both vasodilators. Finally, the renal hemodynamic and natriuretic effects of ACh are probably mediated by prostaglandin release. Circ Res 47: 839-844, 1980

INTRARENAL infusion of vasodilator substances (e.g., acetylcholine or bradykinin) normally is associated with an increase in urinary sodium excretion. Several mechanisms have been proposed to explain this natriuretic response, such as a direct effect of the vasodilator on tubular sodium reabsorption (Parmalee and Carter, 1968; Stein et al., 1972), hemodynamic changes in the Starling forces operating at the postglomerular capillary level (Earley and Friedler, 1966), changes in the intrarenal interstitial pressure (Marchand et al., 1977), or an increase in the medullary blood flow which dissipates the medullary hypertonicity (Earley and Friedler, 1965, 1966).

In contrast with other vasodilators, intrarenal administration of secretin produces a dose-related increase in renal blood flow without a significant rise in urinary sodium excretion (Marchand et al., 1977). A recent study by Marchand et al. (1977) showed that the intrarenal infusion of secretin in the dog was associated with a significant increase in the peritubular capillary hydrostatic pressure and an unchanged interstitial pressure. On the other
hand, acetylcholine produced a marked increase in both these parameters. This suggested that the lack of natriuresis could be explained by the failure of secretin to increase the intrarenal interstitial pressure. Recent experiments in the dog showed that secretin did not augment the renal papillary plasma flow (PPF), suggesting, at least in this animal, that alterations in medullary hemodynamics were of importance in the natriuresis of drug-induced vasodilation (Fadem et al., 1977).

Until now, the effects of secretin on the relationship between total renal and medullary hemodynamics and sodium excretion have not been studied in the rat. In this study, we have examined this relationship with secretin, in comparison to acetylcholine. In addition, renal function and papillary plasma flow measurements were undertaken with both vasodilators after the administration of a prostaglandin synthesis inhibitor because of the suggestion that renal prostaglandin release controls, at least in part, medullary blood flow in the rat (Solez et al., 1974).

Methods

Clearance Studies

All studies were performed in male Wistar rats, weighing 270–330 g and anesthetized with Inactin (50 mg/kg, ip) (Promonta). A tracheotomy was performed and one carotid and one jugular vein were cannulated. For infusion of the vasodilators, a peritoneal catheter was inserted into the aorta at the level of both renal arteries and kept open with an isotonic solution of 10% inulin. A polyethylene tube (PE50) was threaded through the left iliac artery into the aorta at the level of both renal arteries and kept open with an isotonic Ringer's solution containing 10% inulin and 1.7% PAH was infused intravenously at a rate of 0.02 ml/min. The bladder was catheterized through an abdominal incision. Blood pressure was monitored throughout the experiment. At the start of the experiment, an isotonic Ringer's solution containing 10% inulin and 1.7% PAH was infused intravenously at a rate of 0.05 ml/min throughout the course of the experiment.

An equilibration period of 60 minutes was allowed prior to three 10 minute control clearance collections. Four groups of clearance studies were performed.

Group I, acetylcholine studies ($n = 10$). After termination of the control collections, acetylcholine (ACh) (4 μg/kg per min) was infused into the aorta at a rate of 0.02 ml/min. Fifteen minutes after the initiation of this infusion, three 10-minute experimental clearance collections were started.

Group II, secretin studies ($n = 14$). The same protocol was applied as in Group I, but secretin (Karolinska Institutet) dissolved in saline immediately before infusion was administered into the aorta in a dose of 75 mU/kg per min. The experimental clearance collections were started 15 minutes after the initiation of the secretin infusion.

Group III, meclofenamate plus acetylcholine ($n = 8$). Meclofenamate (Parke-Davis & Co.) dissolved in isotonic saline was given intravenously in a dose of 5 mg/kg body weight over 5 minutes just after the termination of the control clearance collections. Fifteen minutes later, the intra-aortic infusion of acetylcholine was started and the experimental clearances were obtained as in group I.

Group IV, meclofenamate plus secretin ($n = 7$). Fifteen minutes after the administration of meclofenamate, an intra-aortic infusion with secretin was commenced and the experimental clearances obtained as in group II. It has been demonstrated previously that meclofenamate, like other non-steroidal anti-inflammatory agents, such as indomethacin, or RO 20 - 5720, has quantitatively similar effects on renal hemodynamics and urinary prostaglandin excretion (Venuto et al., 1975; Kirschenbaum and Stein, 1976).

Urine samples were collected in preweighed vials and the urine volume was determined gravimetrically. Blood samples were collected from the carotid artery at the beginning and the end of the control and the experimental periods for inulin, PAH, sodium and hematocrit determinations. The clearance results presented are the mean of three such 10-minute collections.

Plasma and urine inulin and PAH concentrations were determined by the anthrone method (Fuhr et al., 1955) and by the method of Harvey and Broth-ers (1962), respectively. The sodium concentrations in urine and plasma were measured with a Klin flame photometer (Beckman Instruments). The urine osmolality was determined with an osmometer (Advanced Instruments).

Papillary Plasma Flow Measurements

Papillary plasma flow (PPF) was determined by a modification of the original method of Lilienfeld et al. (1961). The technique has been described previously in detail (Lameire et al., 1978).

The kidneys were exposed through a midline abdominal incision, and loose non-occlusive silk ties were placed around the renal pedicles. A microscope was used to visualize the left renal surface which was illuminated by a fiberoptic light source. A solution of $^{125}$I-albumin (25 μCi/ml; I.R.E.) and lissamine green in 0.9% NaCl was infused into the jugular vein at a rate of 0.5 ml/min with a two-syringe reciprocal action pump (Harvard Apparatus). At the appearance of the dye in the left kidney, a continuous arterial blood collection from the carotid artery was commenced at the same rate as the albumin infusion. The $^{125}$I-albumin was allowed to circulate thorough the kidney for 10, 12, 15, 20, and 30 seconds.

At the conclusion of the time interval under study, the renal circulation was interrupted by pulling the ties around the renal pedicles, and the arterial collection was terminated. The kidneys were removed with the pedicle ties intact, and the papillae were dissected out of the medulla. The papillae, cleaned from adherent blood, were placed...
in capped, preweighed counting vials. The arterial sample was allowed to run from the PE tubing, in which it had been collected, into a previously hep-arinated microcentrifuge tube. The sample was cen-trifuged and 50-μl aliquots of plasma were counted in duplicate. The radioactivity of the plasma sample and the papillae was measured (Berthold Auto-matic Gamma Changer, BR5) (Lab. Berthold) and expressed as counts/min per ml of plasma and counts/min per 100 g of papilla, respectively. The volume of distribution (Vt) is obtained by dividing counts per 100 g papilla by counts per milliliter plasma. When Vt is plotted against the circulation time for each experimental measurement, the relationship is linear for up to 30 seconds (Fig. 1). For circulation times less than 30 seconds, papillary plasma flow can be calculated using the formula

\[
\text{PPF (ml/min per 100 g) = } \frac{\text{[cpm/100 g tissue]} - \text{[cpm/ml plasma]}}{\text{[(60 sec/min)/circulation time (sec)]}}
\]

All PPF measurements were performed at a transit time of 15 seconds. Since only one determination of PPF can be performed per individual animal, five groups of animals of approximately the same weight were used. Papillary plasma flows were determined in 13 control rats 60-90 minutes after the intra-aortic infusion of isotonic Ringer's solution at a rate of 0.02 ml/min (group I).

In 18 rats, PPF was measured 30-40 minutes after the infusion of either acetylcholine (n = 10) (group II) or secretin (n = 8) (group III). In groups IV and V, PPF was measured in meclofenamate-treated animals (5 mg/kg), 30-40 minutes after infusion with either acetylcholine (n = 8) or secretin (n = 9). In all these PPF studies, the same doses of both vasodilators were used as in the clearance studies.

The data were analyzed by the Wilcoxon test for paired and unpaired observations, and all results are presented as the mean ± SE. A p value less than 0.05 was considered to be significant.

Results

Clearance Studies

The effects of both vasodilators on arterial blood-pressure (BP), kidney function, absolute and fractional sodium excretion, and urinary osmolality are shown in Table I.

During acetylcholine infusion, a significant increase in both glomerular filtration rate (GFR) (∝ < 0.05) and effective renal plasma flow (ERPF) (∝ < 0.01) was noted. A mean increase of 2.11 ml/min in ERPF was observed. Absolute and fractional excretion of sodium increased, and there was a significant fall in urine osmolality. The minor decrease in systemic blood pressure was not significant.

During secretin infusion, no significant changes in GFR or in blood pressure were observed. However, a significant increase in PAH clearance from 5.98 ± 0.38 ml/min to 8.19 ± 0.43 ml/min was present. Despite this increasing ERPF, no change in absolute or fractional excretion of sodium was noted under secretin infusion. The urine osmolality under secretin infusion did not differ from the control urine osmolality.

Table 2 summarizes the effects of a pretreatment with meclofenamate on kidney function under acety-lcholine and secretin administration. In contrast, with the effects of acetylcholine in untreated rats, the increase in both GFR and ERPF was blocked by pretreatment with meclofenamate. Although an almost 2-fold increase in absolute and fractional excretion of sodium was observed, these changes were not significant. In these rats, no significant fall in urine osmolality occurred.

The experiments in which the effects of meclofenamate on kidney function under secretin infusion were studied show changes completely comparable with the results obtained with secretin alone. There was a significant rise in PAH clearance from 6.09 ± 0.27 to 7.69 ± 0.32 ml/min (∝ < 0.01) while all other parameters remained unchanged.

Despite a comparable increase in ERPF, it is apparent that the increase in absolute and fractional sodium excretion is much greater after acetylcholine compared to secretin. The effects of acetylcholine on kidney function and sodium excretion were almost completely blocked by pretreatment of the animals with the prostaglandin synthesis inhibitor meclofenamate.

Papillary Plasma Flow Measurements

Figure 2 shows the individual results obtained for both kidneys in control animals and in the acetylcholine-and secretin-infused animals. The mean papillary plasma flow for both kidneys after secretin was 66 ± 2.75 ml/min per 100 g tissue.
TABLE 1  Effects of Acetylcholine and Secretin on Renal Function

<table>
<thead>
<tr>
<th></th>
<th>BP (mm Hg)</th>
<th>Hct (%)</th>
<th>GFR (ml/min)</th>
<th>PAHCl (ml/min)</th>
<th>UN.V (µEq/min)</th>
<th>FEox (%)</th>
<th>Uosm (mOsm/kg)</th>
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<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td>C</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>112 ±4</td>
<td>105 ±3</td>
<td>49 ±0.6</td>
<td>47 ±0.5</td>
<td>1.96 ±0.11</td>
<td>2.30 ±0.22</td>
<td>5.81 ±0.52</td>
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<td>(n = 10)</td>
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<tr>
<td>Secretin</td>
<td>113 ±3</td>
<td>111 ±4</td>
<td>50 ±0.7</td>
<td>49 ±0.6</td>
<td>2.05 ±0.12</td>
<td>2.10 ±0.38</td>
<td>5.98 ±0.43</td>
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<td>(n = 14)</td>
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Abbreviations: BP, blood pressure; Hct, hematocrit; GFR, glomerular filtration rate; PAHCl, para-amino-hippurate clearance; UN.V, absolute urinary sodium excretion; FEox, fractional urinary sodium excretion; Uosm, urinary osmolality; C, control period; E, experimental period.

* P < 0.05; † P < 0.01.

Although this was significantly greater than control (50 ± 3.16 ml/min per 100 g) the increase in papillary plasma flow after acetylcholine was even greater as the mean PPF rose to 90 ± 3.5 ml/min per 100 g.

Figure 3 depicts the effects on papillary plasma flow of both vasodilators in rats pretreated with meclofenamate. No effect of meclofenamate on PPF after secretin infusion was noted.

In contrast, meclofenamate appears to block substantially the medullary vasodilation by acetylcholine, as after meclofenamate the mean PPF is only 64 ± 2.3 ml/min per 100 g compared to 90 ± 3.5 ml/min per 100 g in untreated animals.

**Discussion**

These studies in the rat confirm previous results obtained in the dog, showing that secretin increases the effective total renal plasma flow without a substantial effect on urinary sodium excretion. In contrast, acetylcholine, like many other renal vasodilator substances, produces a concomitant renal vasodilation and increase in natriuresis. One of the mechanisms that has been proposed to explain this different behaviour is the failure of secretin to influence the intrarenal interstitial pressure (Marchand et al., 1977).

The possibility that lack of alteration in papillary plasma flow might be involved in the natriuretic defect seen with secretin was investigated in the present studies, and the renal effects of secretin were compared to the effects of acetylcholine, a vasodilating and natriuretic substance. Plasma flow in the papilla was measured with the accumulation technique originally described by Lilienfield et al. (1961) and subsequently modified by Soble et al. (1974) and Ganguli and Tobian (1974). The theoretical and practical aspects of this method have been discussed in detail by each of these investigators. Although the control values for the PPF measurements reported in the present studies show a relatively wide variation with a standard error of the mean of 3.16 ml/min per 100 g, the differences between groups are easily demonstrated and there is excellent agreement between values for the right and left kidney in the same animal. As is shown in Figure 2, PPF was significantly higher in the acetylcholine-infused kidneys compared with the secretin-infused kidneys. In addition, a consistently lower urinary osmolality was observed with acetylcholine compared to secretin. This latter effect is to be expected since an increase in papillary plasma flow could lead to a fall in urine osmolality by removing more solute from the medullary interstitium than is added to it (Berliner et al., 1958; Thurau, 1964).

An increase in medullary plasma and blood flow has been proposed to influence net sodium reabsorption in the medullary tubular structures in the so-called "medullary wash-out" theory (Earley and Friedler, 1965, 1966). Dissipation of the medullary hypertonicity would decrease passive water loss from descending limbs of Henle's loop, increase volume flow through ascending limbs, and reduce absolute solute reabsorption by the ascending limbs. Since, compared to acetylcholine, secretin has only a trivial influence on papillary plasma flow, it is conceivable that the lack of this hemodynamic

TABLE 2  Effects of Acetylcholine and Secretin on Renal Function in Meclofenamate-Pretreated Rats

<table>
<thead>
<tr>
<th></th>
<th>BP (mm Hg)</th>
<th>Hct (%)</th>
<th>GFR (ml/min)</th>
<th>PAHCl (ml/min)</th>
<th>UN.V (µEq/min)</th>
<th>FEox (%)</th>
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<td>C</td>
<td>E</td>
<td>C</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>111 ±3</td>
<td>108 ±4</td>
<td>50 ±0.7</td>
<td>49 ±0.6</td>
<td>2.15 ±0.14</td>
<td>2.17 ±0.12</td>
<td>6.15 ±0.12</td>
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<td>(n = 8)</td>
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<tr>
<td>Secretin</td>
<td>112 ±4</td>
<td>113 ±4</td>
<td>48 ±0.8</td>
<td>47 ±0.6</td>
<td>2.07 ±0.13</td>
<td>1.98 ±0.11</td>
<td>6.09 ±0.27</td>
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<td>(n = 7)</td>
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Abbreviations: see Table 1.

* P < 0.01.
FIGURE 2 Summary of papillary plasma flow (PPF) data. PPF values are shown for control rats as well as rats infused with either acetylcholine or secretin. Individual data for right (R) and left (L) kidneys are given.

The minimal papillary vasodilation observed with secretin is the reflection of the absence of inner cortical flow redistribution, an alternative second hypothesis can be offered to explain the dissociation between renal blood flow and sodium excretion with secretin. It previously has been demonstrated that acetylcholine-induced vasodilation causes no redistribution of glomerular filtrate (Stein et al., 1971b). If glomerular filtrate distribution is unchanged while plasma flow is disproportionately increased in juxtamedullary nephrons, the filtration fraction would be reduced to a greater extent in the deep nephrons. Studies on acetylcholine and bradykinin administration by Stein et al. (1972) have indeed shown a greater fall in filtration fraction in deep nephrons, presumably leading to a lower oncotic pressure in their postglomerular circulation. Consequently, this would result in a greater fall in sodium reabsorption in the deep nephrons and enhanced sodium excretion. Since secretin, despite its vasodilatory effect, has no influence on cortical blood flow distribution, a more pronounced change in deep nephron filtration fraction and increased sodium excretion cannot be expected.

In the second part of this study, an attempt was made to elucidate further the mechanism of the renal and medullary vasodilation of acetylcholine, by repeating the experiments after administration of meclofenamate, a prostaglandin synthesis inhibitor. The dose of meclofenamate used in this study was similar to or even higher than that used in numerous other studies in which the role of endogenous prostaglandins was studied in isolated as well as in intact kidneys (Lonigro et al., 1973; Kirschbaum and Stein, 1976; Scherer et al., 1978).

In a recent study from this laboratory, intravenous administration of meclofenamate, 5 mg/kg, reduced the prostaglandin E$_2$ and F$_{2\alpha}$ urinary excretion rate in water and Ringer's loaded conscious rats by more than 50% and blunted completely the rise in prostaglandin excretion normally associated with an extracellular volume expansion (Lameire et al., 1980).

Previous studies have indicated that administration of renal vasodilating substances like bradykinin was associated with renal prostaglandin release (McGiff et al., 1972). It has also been shown that prostaglandin inhibition produces a fall in papillary plasma flow in the rat (Solez et al., 1974). In fact, in the present study, the rise in both the effective total renal and medullary plasma flow with acetylcholine was inhibited to a great extent by meclofenamate. The natriuretic response in these animals also was markedly attenuated in comparison to the natriuresis observed in the intact animals.

In contrast, no effect of the prostaglandin inhibitor on either total or medullary plasma flow was observed in the secretin-infused rats. As far as we know, data on the effects of acetylcholine on prostaglandin secretion are not available in the litera-
ture. The urinary prostaglandin measurements in anesthetized animals post-laparotomy are extremely difficult to interpret due to the dramatically high basal prostaglandin synthesis associated with this state, and were therefore not obtained in this study (Scherer et al., 1978). However, preliminary studies in our laboratory in which the urinary excretion of PGE 
2 and PGF 
2o were studied after either acetylcholine or secretin in conscious rats, show that the PG excretion is elevated after administration of the former but remains unaltered after the latter drug (N. Lameire and M. Korteweg, unpublished observations). The same results for PGE 
2 excretion after secretin were obtained in the anesthetized dog (Fadem et al., 1977).

Taken together with the present study, these results do not suggest that prostaglandins are a major determinant of the vasodilatory effects of secretin.

In summary, the present studies confirm in the rat the dissociation between the renal vasodilation and the natriuresis obtained with secretin. They further suggest that the absence of a natriuretic response observed with this drug is related to its inability to increase medullary blood flow substantially. Finally, the results are compatible with the view that prostaglandin release is of importance in the vasodilating and natriuretic responses obtained with acetylcholine, and possibly other renal vasodilating substances.

Acknowledgments

We wish to express our appreciation to L. De Torck and C. Vandenberghe for secretarial assistance.

References

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_Circ Res._ 1980;47:839-844
doi: 10.1161/01.RES.47.6.839

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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