VASODILATOR PROPERTIES OF ETHACRYNIC ACID
IN THE PERFUSED DOG KIDNEY

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(Received 4 August 1969)

SUMMARY

1. Ethacrynic acid reduces renal vascular resistance of the isolated dog kidney.
2. Ethacrynic acid induced natriuresis can occur despite stabilization of renal
   blood flow, but augmentation of blood flow increases the natriuretic response.
3. Vasodilatation and natriuresis after ethacrynic acid administration occurred
   together in time and were not separable. The onset and peak natriuretic and vasodilator
   responses frequently coincided in time.
4. Ethacrynic acid induced vasodilatation could be inhibited by loss of body fluid.
   The rise in resistance could be avoided by adequate volume replacement and by
   prevention of volume depletion.

Ethacrynic acid (2,3-dichloro-4-(2-methylene-butyryl)-phenoxyacetic acid) is a potent natriuretic agent. Many of the pharmacologic properties of ethacrynic acid have been previously described. Beyer et al. (1965) has comprehensively described the natriuretic properties of this compound. Other workers have attempted to define the site of action of ethacrynic acid within the nephron. Goldberg et al. (1964) and Earley & Friedler (1964) both concluded that ethacrynic acid has an important site of action in the loop of Henle as judged by its interference with renal diluting and concentrating mechanisms. Birtch et al. (1967) studied the redistribution of renal blood flow following ethacrynic acid administration. He reported a decrease in outer medullary and juxtamedullary cortical blood flow, while outer cortical flow was increased following ethacrynic acid administration. The present study further examines the vasoactive properties of ethacrynic acid in the in vivo perfused dog kidney, under conditions of either constant flow or constant pressure. The time course of vasodilatation was compared with the time course of the natriuretic response in an attempt to determine the role of vasodilatation in the natriuretic response.

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Studies were performed on mongrel female dogs (approximately 20 kg) anaesthetized intravenously with sodium pentobarbitone. The induction dose was 30 mg/kg and light anaesthesia was maintained by intermittent intramuscular injections, preserving the lateral canthal reflex. Food was withdrawn 12–18 hr before an experiment and the dogs were allowed unlimited water.

The brachial artery was cannulated for continuous pressure recording. Renal function measurements were made using a sustaining infusion of inulin and PAH sufficient to maintain plasma levels of approximately 30 mg/100 ml and 3 mg/100 ml, respectively. In addition, a sustaining solution of isotonic 2.5% mannitol in 0.45% saline solution was given at a rate of 3 ml/min. A mid-line lower abdominal incision was made, the ureters exposed and cannulated with polyethylene tubing. A left flank incision was then made and dissection carried out to expose the aorta and left renal artery. In most instances this dissection was accomplished without entering the peritoneum. Regional lumbar vessels (usually two pairs) were clipped and divided. Next, the aorta was clamped and severed below the renal artery and a cannula inserted into the severed aorta. This cannula (Fig. 1) had two collars which could be separated or telescoped upon one another. After insertion, the distal collar was tied in place. The proximal collar was then advanced until it lay between the left and right renal arteries and then tied in place. Thus, an aortic pouch containing only the left renal artery was created between the two
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Collars of the cannula (Fig. 1). Blood taken from the left carotid artery was delivered via the cannula to the pouch with a roller type pump (CINCO, Wakefield, Mass.). The right kidney remained perfused by the systemic circulation and served as a control. In some instances, the left and right renal arteries were too close together to allow insertion of the proximal collar between them. In such cases, the collar was advanced beyond the right renal artery and the right renal artery clamped.

Continuous pressure readings were taken from the pouch and brachial arteries and recorded on a multi-channel Sanborn direct recorder (Model 350–110B) utilizing Statham transducers (Model P23 Db). Electrical damping was used to obtain the mean arterial pressure (MAP). Perfusion flow rate was monitored by an electromagnetic flow meter (Medicon Model E300b).

After completion of surgery, a 30 min equilibration period was allowed to ensure stability of systemic and pouch pressures. During this time, renal blood flow was adjusted by the perfusion pump to keep mean pouch pressure 10–15 mmHg less than systemic pressure (between 100–150 mmHg).

There were 3 modes of ethacrynic acid administration:

1. Bolus injection (2–10 mg) over a 30 sec period directly into the perfused kidney via the perfusion tubing.
2. Bolus injection (2–10 mg) systemically via the intravenous route.
3. Constant infusion at a rate of 1 mg/min into the perfused kidney via the perfusion tubing.

Ethacrynic acid given by these modes of administration was given under two experimental conditions, perfusing either at constant flow or at constant pressure. Under conditions of constant pressure, flow was continuously adjusted to ensure a constant renal pouch pressure. Before a second bolus of ethacrynic acid was given, renal resistance was allowed to return to its control preinjection value or a stable new value.

Prior to ethacrynic acid administration, two 10-min control urine collections were taken. All plasma samples were obtained at the mid-point of the urine collection periods. Following ethacrynic acid administration, urine was collected at 1 min intervals for the first 10 min and then at 10 min intervals for 50 min.

Sodium and potassium concentrations were measured by flame photometry using lithium as an internal standard. Osmolality was determined using an Advanced Osmometer. Chloride determinations were performed using an automatic Cotlove titrator. PAH clearance ($C_{PAH}$) was used as an index of renal blood flow; inulin clearance ($C_{In}$) was used as an index of glomerular filtration rate (GFR). PAH was analysed by the method of Brod & Sirota (1948) as modified by Smith (1951) using a tungstate filtrate. Inulin in urine and plasma was determined by the method of Schreiner (1950).

Resistance cannot be measured by direct means and must be calculated from measurements of blood flow and pressure gradient (Resistance = Pressure drop/Flow rate). Resistance in our experiments was calculated by dividing pouch pressure by renal blood flow. The values are expressed in peripheral resistance units (PRU) or mmHg min ml⁻¹. In our calculations we did not correct for renal vein pressure (Earley & Daugharty, 1969; Hardin, Scott & Haddy, 1960). An alternative would be to assume a renal vein pressure of 8–10 mmHg (Gomez, 1941). Significant changes in renal vein resistance most probably occur proximal to sites easily approached with a catheter in the renal vein. Further, the magnitude of resistance changes observed are unlikely to be explained by changes in renal vein resistance.
RESULTS

Physiology of the perfused kidney

Measurements of the function of the perfused kidney were contrasted with those from the contralateral systemically-perfused kidney. Data were obtained using thirty-three paired controlled periods in 10 dogs. When allowances were made for pressure matching, it was found that the perfused kidney behaved quantitatively and qualitatively like the systemically-perfused kidney with regard to PAH and inulin clearance, per cent sodium reabsorbed, urine volumes and filtration fraction (Fig. 2). In the perfused kidney, mean control resistance at constant pressure was 0.75 PRU and 0.82 PRU at constant flow.

Effects of ethacrynic acid on renal resistance

The first response to ethacrynic acid injection was a rise in renal resistance. The rise occurred within the first few minutes and was promptly followed by a decline in renal resistance, the onset of decline occurring within 2–5 min after injection and reaching minimum resistance on average 10 min after ethacrynic acid administration. After a single bolus injection, renal resistance returned toward control values within 20–40 min. Similar patterns of response in renal resistance occurred with ethacrynic acid doses ranging between 2 and 10 mg given into the pouch or systemically by the intravenous route. The doses of ethacrynic acid administered in these experiments did not lead to a fall in systemic blood pressure.

Perfusing at constant pressure, renal resistance fell on the average from 0.75 PRU (control) to 0.56 PRU (minimum value) (P < 0.01). A fall in resistance was seen following ethacrynic acid bolus injection in each of seven experiments performed at constant pressure. Perfusing
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at constant flow, the average fall in resistance was from 0.82 PRU (control) to 0.52 PRU (minimum value) \( (P<0.01) \). At constant flow, a fall in renal resistance was produced by ethacrynic acid bolus injection in each of nine experiments performed. These values represent a total of twenty-three observations in fifteen dogs.

Fig. 3 depicts a representative experiment illustrating the action of ethacrynic acid on renal resistance at constant flow. In this experiment, 6 mg of ethacrynic acid was given into the pouch via perfusion tubing. There was an early transient rise in renal resistance followed by an immediate fall, the onset of decline occurring within 2 min and reaching a minimum value

5 min after injection. In this experiment, renal resistance returned toward control levels within 30 min after ethacrynic acid injection. No change in systemic blood pressure was observed. A similar pattern of response was seen at constant pressure following a 10 mg ethacrynic acid bolus directly into the kidney (Fig. 4).

**Natriuretic compared with vasodilator responses**

The time course of the vasodilatory and natriuretic actions of ethacrynic acid was compared in twenty-three experiments. The onset of decline in resistance and the onset of natriuresis occurred simultaneously in all experiments. Natriuresis and vasodilatation both occurred within several minutes after injection of ethacrynic acid. Fig. 4 illustrates the coincident fall in resistance and onset of natriuresis while perfusing at both constant flow and pressure. The average peak natriuresis occurred within 15 min at a time when resistance was usually at its minimum value. In several experiments, a separation of resistance and natriuresis was observed, peak natriuresis occurring when renal resistance was already rising and returning toward control value.

An increased absolute rate of natriuresis was usually observed perfusing at constant pressure.
compared to perfusing at constant flow (Fig. 4). This was observed in four of six experiments following administration of identical doses of ethacrynic acid directly into the kidney under conditions of both constant flow and constant pressure. This increased rate of natriuresis observed at constant pressure is most likely explained by the changes in inulin and PAH clearances observed under the two experimental conditions. At the time of minimum resistance under conditions of constant flow, there was usually a decline in inulin and PAH clearances (Table 1). At constant pressure, where increased blood flow was necessary to maintain the constancy of pressure, there was usually an increase in PAH and inulin clearances at the time of minimum resistance.

Thus, an increase in renal blood flow seems to augment the natriuretic response. The experiments performed at constant flow, however, illustrate that an increase in renal blood flow is not necessary for some natriuretic response to occur.

Effect of body fluid depletion upon ethacrynic acid induced renal vasodilatation

In some of our experiments, renal resistance rose spontaneously following bolus ethacrynic acid administration despite persistent natriuresis. To investigate a possible role of fluid loss in
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Table 1. Mean values (±SEM) for inulin and PAH clearances and PAH extraction following ethacrynic acid

<table>
<thead>
<tr>
<th></th>
<th>Average value</th>
<th>Value at minimum resistance</th>
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<tbody>
<tr>
<td><strong>Inulin clearance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant flow (ml/min)</td>
<td>27±4</td>
<td>23±3</td>
</tr>
<tr>
<td>Constant pressure (ml/min)</td>
<td>25±3</td>
<td>31±6</td>
</tr>
<tr>
<td><strong>PAH clearance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant flow (ml/min)</td>
<td>92±9</td>
<td>70±10</td>
</tr>
<tr>
<td>Constant pressure (ml/min)</td>
<td>85±6</td>
<td>91±6</td>
</tr>
<tr>
<td><strong>Renal blood flow</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant flow (ml/min)</td>
<td>197±14</td>
<td>197±14</td>
</tr>
<tr>
<td>Constant pressure (ml/min)</td>
<td>190±14</td>
<td>252±16</td>
</tr>
<tr>
<td><strong>PAH extraction ratio (calculated)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant flow (ml/min)</td>
<td>0.82±0.04</td>
<td>0.60±0.06</td>
</tr>
<tr>
<td>Constant pressure (ml/min)</td>
<td>0.71±0.05</td>
<td>0.57±0.05</td>
</tr>
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modifying the ethacrynic acid induced vasodilatory response, experiments were performed with a continuous ethacrynic acid infusion (0.5–1.0 mg/min directly into the perfused kidney). The response of renal resistance to continued ethacrynic acid infusion was similar in magnitude and course to that seen following ethacrynic acid bolus. This prolonged infusion of ethacrynic acid was often associated with a prolonged fall in renal resistance, illustrating that the vasodilatation induced by ethacrynic acid is not a transient phenomenon (Fig. 5). In four of seven experiments performed with a continuing ethacrynic acid infusion, renal resistance was noted to rise spontaneously. In these experiments, volume depletion was judged to be the factor mediating the rise in resistance during a continuing ethacrynic acid infusion. Volume depletion was evidenced by a falling rate of natriuresis at the time renal resistance was rising. This decline in natriuresis was more clearly seen in the non-perfused kidney which was not forced to accept a fixed blood flow. A rising haematocrit was also characteristically seen in those experiments where a rise in renal resistance was observed during continued ethacrynic acid infusion.

Volume repletion (of the total volume lost) was thus instituted when renal resistance began to rise during a prolonged ethacrynic acid infusion. In three of four experiments, volume repletion in this fashion was able to reverse the rising renal resistance and vasodilatation was again evident (Fig. 5). To further test the hypothesis that volume depletion was responsible for this rise in renal resistance, a continuous ethacrynic acid infusion was repeated but prevention of volume depletion was attempted. Volumes of Ringer’s lactate were infused intravenously to equal the urinary output (Fig. 6). The concentration of sodium in the Ringer’s lactate was found to be approximately equal to that observed in the urine at peak natriuresis. By preventing volume depletion, it was possible to sustain vasodilatation almost indefinitely and the late rise in resistance was abolished (three of four experiments).
Fig. 5. Effect of volume depletion and repletion on renal resistance, urine volume and urinary sodium excretion.

Fig. 6. Effect of ethacrynic acid infusion and simultaneous volume repletion on renal resistance, urine volume and urine sodium excretion.
DISCUSSION

Diuretic agents have a variable effect upon renal resistance. Hook et al. (1966) reported vasodilatation in the canine dog kidney following intravenous administration of ethacrynic acid and frusemide. Birtch et al. (1967) also reported vasodilatation following ethacrynic acid injection into the renal artery of the dog. Our observations in the perfused dog kidney further confirm that ethacrynic acid is a potent renovasodilator. Injection of frusemide along with two other diuretic agents (meralluride and chlorothiazide) has also been performed in our perfusion model (Fig. 7). Frusemide was also found to be a potent renal vasodilator, while meralluride was found to be a potent renal vasoconstrictor, and chlorothiazide, a mild renal vasoconstrictor. The observations with regard to meralluride and chlorothiazide are consistent with previous reports (Vargas & Cafruny, 1962; Hook et al., 1966). Thus, vasodilatation is not a characteristic response of all diuretic agents. In fact, renovasodilatation appears to be characteristic of those agents that may act at the loop of Henle, as evidenced by their interference with the concentrating mechanisms of the kidney.

Ethacrynic acid-induced renovasodilatation was intimately linked in time to the natriuretic response. The onset of natriuresis and vasodilatation occurred together, although the maximal responses were not always coincident. Vasodilatation without natriuresis, or natriuresis without vasodilatation, were never seen. Both natriuresis and vasodilatation were also seen in the dog kidney perfused at constant flow. Therefore an increase in flow accompanies but is not necessary for ethacrynic acid-induced natriuresis. However, an increase in blood flow may serve

![Graph showing effect of various diuretics on renal resistance.](image-url)

Fig. 7. Effect of various diuretics on renal resistance.
to augment the natriuretic response. This was evidenced by a greater maximal rate of natriuresis seen under conditions of constant pressure as compared to constant flow.

The pattern of an initial brief rise in resistance followed by prolonged vasodilatation was observed by Birtch et al. (1967). Following injection of ethacrynic acid into the renal artery, a 10–15% fall in renal blood flow was immediately observed, followed by a slower 25–30% increase in renal blood flow. The time course of these observations corresponds to the pattern observed in our experiments. Birtch suggested that ethacrynic acid and frusemide may initiate natriuresis by intrarenal redistribution of blood flow. However, it is also possible that the vascular response is secondary to a primary action of ethacrynic acid upon the renal tubules. Our data could support either hypothesis.

Comment might also be made of the relevance of this data to renal blood flow 'autoregulation'. It was noted that renal resistance fell less after ethacrynic acid bolus at constant pressure compared to constant flow, suggesting an attempt at autoregulation responsive to perfusion pressure but not flow. Autoregulation of glomerular filtration was quite incomplete, inulin clearance rising at constant pressure and falling at constant flow, even though perfusion pressure at minimum resistance during constant flow averaged 100 mmHg. This value is said to be within the glomerular filtration autoregulation range (Harvey, 1964; Hardin et al., 1960). Despite the divergence of glomerular filtration rates, the filtration fraction (\(C_{\text{in}}/C_{\text{PAH}}\)) responded to ethacrynic acid almost identically in both cases (increasing from 0.29 to 0.33 at constant flow and 0.29 to 0.34 at constant pressure). This suggests that PAH clearance may have been altered by both ethacrynic acid and flow/pressure distribution since at constant flow PAH clearance fell. The changes in calculated PAH extraction, therefore, are less dramatic at constant pressure than at constant flow. This complex series of changes would seem best explained by haemodynamic redistribution as shown by Birtch, but one must also assume that PAH extraction is probably not an exclusive index of medullary blood flow. It must also be assumed that renal haemodynamics influence the tubular handling of PAH. However, the relationship between vascular and tubular factors in determining renal excretory function must be further explored.

Luddens et al. (1968) reported that the degree of vasodilatation and enhancement of renal blood flow by frusemide was related to the initial resistance to blood flow in the kidney. In this report, initial renal resistance was modified prior to frusemide administration by different intravenous saline and mannitol loads. In our experiments, it is of interest that continued ethacrynic acid-induced vasodilatation was volume-dependent. Volume depletion was seen to reverse the renovasodilatation previously established by ethacrynic acid administration. Thus, a maximal natriuretic response produced by ethacrynic acid would be expected in a subject in a normal or supranormal state of hydration. Maintenance of an adequate plasma volume may indefinitely perpetuate ethacrynic acid-induced vasodilatation and natriuresis. Further work is necessary to determine whether the rise in resistance secondary to volume depletion is mediated by the sympathetic nervous system and whether adrenergic blocking agents can abolish this late rise in resistance.

ACKNOWLEDGMENTS

These investigations were supported in part by the John A. Hartford Foundation Grant 9893, and in part by Public Health Service Research Grant AM-5100-10 from the National Institute of Arthritis and Metabolic Diseases, and in part by the Cardiovascular Grant
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9381–3. R. G. Dluhy held U.S. Public Health Postdoctoral Research Fellowship F2-AM-36, 249. G. L. Wolf was a Life Insurance Medical Research Fellow.

REFERENCES


