In situ Kidney Pressure-Flow-Resistance Relationship During Endotoxin Shock in Dogs.* (31136)

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Few data are available relative to resistance changes in the intact dog kidney during irreversible endotoxin shock. The mechanism of kidney damage following bacteremic shock in man(1) and Gram-negative bacterial endotoxin shock in animals(2) has been largely delineated. Comprehensive studies using dogs have shown that hemodynamic derangement and not a direct nephrotoxic action of endotoxin is responsible for renal damage(2-4). Resistance changes during endotoxin shock have been noted in constant flow(3) perfused dog kidneys and have been inferred from kidney weight changes(4). The present study was undertaken to determine pressure-flow-resistance relationships during irreversible endotoxin shock in natural flow kidneys perfused *in situ* by intact dogs. Particular attention is given to resistance changes during shock.

Materials and methods. Adult mongrel dogs of both sexes were anesthetized intravenously with 30 mg/kg sodium pentobarbital. Heparin sodium, 3 mg/kg, was used as anticoagulant. A polyethylene catheter was placed in the aorta through a femoral artery to measure renal artery orifice pressure (aortic blood pressure) which was recorded using a Statham pressure transducer and Sanborn direct-writing recorder. The left kidney was exposed retroperitoneally through a flank incision. The renal pedicle was minimally disturbed and care was taken not to denervate the kidney. The renal artery was occluded for a few seconds with a nontraumatic clamp during cannulation of the renal vein with appropriately sized plastic tubing. The position of the venous outflow catheter tip was placed lower than the kidney until a slight "flutter" of the renal vein resulted. This assured a mean renal vein pressure near zero mm Hg at all times. Venous blood flowed by gravity into a dextran filled, 100 ml plastic reservoir and was returned to a cannulated femoral vein by a Sigmamotor pump. The kidney was covered loosely with polyvinyl sheeting and external temperature of the kidney was maintained at near 38°C with heat lamps. Blood flow was measured with a graduated cylinder and stopwatch. Renal vascular resistance was calculated by dividing the pressure gradient from renal artery (aorta) to renal vein (zero mm Hg) by renal blood flow.

Pressure and flows were allowed to stabilize and 10-15 minutes of steady-state measurements were obtained. Five dogs were prepared in an identical fashion except that endotoxin was not given and these animals served as controls for the shocked dogs.

Statistical comparisons for differences between the means of animals receiving endotoxin *vs* control dogs receiving no endotoxin were made at each piont. "P" values less than 0.05 were considered significant.

Results. Dog and left kidney weights for the experimental group averaged 10 kg and 27 g, respectively; corresponding values for control dogs averaged 13 kg and 33 g, respectively.

Fig. 1 shows renal hemodynamic changes following systemic intravenous injection of a lethal amount of *E. coli* endotoxin. Starting values of the two groups were not significantly different ($P>.05$). Immediately following intravenous endotoxin injection renal artery pressure and blood flow fell precipitously while renal vascular resistance increased markedly ($P<.01$, $<.01$ and $<.05$, respectively). While considerable variation of individual response magnitude was noted, directional changes of each parameter indicated by the means occurred in every experiment. These changes were maximum at 2-5 minutes. From this point to 30 minutes all parameters

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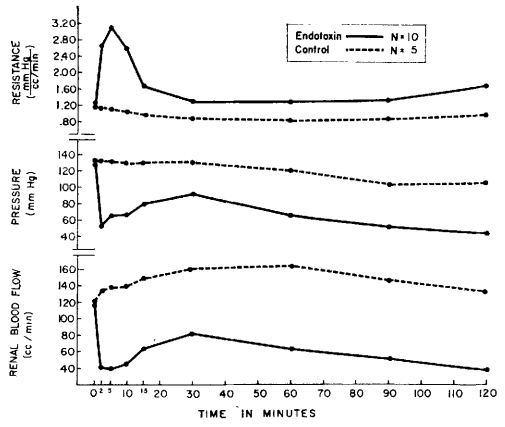

FIG. 1. *In situ* kidney. Endotoxin given intravenously as a bolus injection at "0" time. Resistance = total renal vascular resistance; pressure = renal artery orifice pressure (aortic blood pressure).

tended to return towards control level. At 30 minutes after endotoxin injection renal perfusion pressure and renal blood flow were still considerably below control ($P<0.05$); however, renal resistance in each experiment had returned to near control level by this time and mean resistances of control and experimental kidneys were not significantly different ($P>.05$). From 30-90 minutes renal perfusion pressure and blood flow again decreased and renal resistance remained at a level not different from control ($P>.05$). From 90-120 minutes after endotoxin mean renal blood flow and mean perfusion pressure decreased while mean renal resistance again increased. However, this resistance increase was not statistically significant although it occurred in 7 of 10 experiments ($P>.05$).

Discussion. The present study is of particular interest in that almost immediately after attaining a peak increase following endotoxin injection, renal vascular resistance began to decline towards pre-endotoxin level, and reached control by 30 minutes. The early increase in resistance appears to result largely from sympatho-adrenal discharge initiated by hypotension and activation of the barostatic mechanism; it is totally blocked by pretreatment with phentolamine in kidneys in which blood flow is maintained constant(3). In the present study, since pressure fell markedly, passive constriction probably also contributed to the overall resistance increase which was superimposed on the normal tendency of renal autoregulation(5). The early resistance increase is qualitatively similar to that observed in constant flow perfused kidneys following systemic endotoxin injection in dogs, although in this study peak resistance increase was at 10 minutes(3). However, changes in resistance from 10 to 30 minutes differ in one major respect. Resistance in constant flow perfused kidneys decreased during this period, but never reached control, pre-endotoxin level; renal resistance was an average of 35% above control at 30 minutes after endotoxin(3). This difference is also similar to data obtained in natural pressure-flow perfused *vs* constant flow perfused kidneys following hemorrhagic hypotension in dogs using a preparation similar to that employed in the present study(6). Haddy, Scott and Molnar first observed an increase in renal resistance which decreased to near control by about 30 minutes after hemorrhage(6). However, in constant flow perfused kidneys, renal resistance decreased from the initial peak elevation but remained at a value well above control throughout the approximately 30-minute post-hemorrhage period(6). This difference between natural and constant flow flidneys might be explained on the basis of a local increase of vasodilator metabolites which could occur when flow was severely depressed(7); however, this would be less likely to occur with flow constant. Hence, a severely reduced renal blood flow might lead to a local build-up of vasodilator metabolites which antagonize renal vasoconstriction and lower renal resistance to blood flow. Other factors may also contribute to the secondary decrease of renal resistance. Renal vascular responsiveness to angiotensin II is greatly reduced during endotoxin shock; however, the action of the catecholamines and histamine is not appreciably altered(8). While little information is available as to angiotensin release during endotoxin shock, catecholamine blood concentration is seen to increase strikingly immediately after endotoxin injection but subsequently falls to a level which is still above normal during the first hour after endotoxin(9). This alone would lead to parallel renal resistance changes. An apparent "resetting" of the barostatic con-

trol mechanism caused by endotoxin acting on the carotid sinus also has been noted(10). Histamine release, particularly during the early phase of endotoxin shock, has been demonstrated(11) and would tend to antagonize the constrictor action of released vasoconstrictors. These factors, plus a local build-up of vasodilator metabolites, apparently exactly balance constrictor forces until the animal deteriorates beyond some critical point.

Finally, it is of interest that up to 90 to 120 minutes following a lethal, overwhelming dose of endotoxin, except during the very early phase, renal blood flow does not appear to be reduced as much as would be expected from the precarious state of the animal. And it is only near death that flow is actually reduced out of proportion to renal perfusion pressure in a majority of kidneys while other kidneys maintain a low resistance even when the dogs's arterial pressure is extremely low.

Summary. Experiments were completed in anesthetized, mongrel dogs. Kidneys were exposed retroperitoneally through a flank incision to facilitate direct blood flow measurement from a cannulated renal vein. Perfusion was through the intact renal artery of the dog. Renal vascular resistance increased markedly as perfusion pressure and renal flow decreased to low levels by 2 minutes after intravenous endotoxin injection. By 30 minutes, perfusion pressure and flow were still low but renal resistance was at pre-endotoxin level where it remained until 90-120 minutes after endotoxin at which time resistance either increased or remained near control level. Renal perfusion pressure and blood flow were both very low at this point. These data indicate that during irreversible endotoxin shock, except for the very early phase, renal blood flow is not decreased out of proportion to renal perfusion pressure. During the latter period of shock, at extremely low arterial pressure, renal resistance may or may not be elevated.

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Fractionation of H-2 Antigenic Specificities in A/Sn Mice.* (31137)

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The different techniques for subcellular fractionation which have been used for the study of H-2 antigens in mice have shown these to be associated with cellular membranes (1,2,3,4,5). Extraction of these antigens from the membranes presents some difficulties and the "molecular" characteristics of the fractions obtained depend greatly upon the method used for preparation of the antigen. Kandutsch and Stimpfling(6) are the only authors to report a method of extraction and purification of H-2 antigens using detergents (Triton X 100) which has resulted in maintenance

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