Renal Response to Electrical Stimulation in the Septum and Diencephalon of Rabbits

By Amin N. Jurf and William D. Blake

ABSTRACT

The septum and diencephalon were electrically stimulated with stereotaxically placed bipolar, concentric electrodes in pentobarbital anesthetized rabbits with one kidney acutely denervated. Transient changes (30-60 seconds) in renal blood flow and urine flow were estimated with electromagnetic flowmeters and drop recorders and persistent changes (20-30 minutes) by clearance techniques. Hypothalamic stimulation, particularly in the perifornical region, caused increased blood pressure, reflex bradycardia (blocked by atropine), and intense renal vasoconstriction in the innervated kidney with a latency of about 2 seconds. In the denervated kidney renal blood flow increased with the rise in blood pressure but after 10-15 seconds decreased, possibly in response to catecholamines released from the adrenal glands. Clearance studies during hypothalamic stimulation showed persistent decreases in renal blood flow, glomerular filtration, maximal tubular transport of glucose and para-aminohippurate, and water and electrolyte excretion in the innervated kidney. The parallel decreases in the maximum tubular transport of glucose and glomerular filtration rate suggest nephron closure rather than flow redistribution as the major component of the response. Ventral thalamic and septal stimulation decreased blood pressure and renal blood flow in the denervated kidney to about the same extent. Ventral thalamic stimulation also decreased renal blood flow in the innervated kidney whereas septal stimulation transiently increased renal blood flow, suggesting inhibition of existing sympathetic vasoconstriction (not blocked by atropine). In clearance studies, effects of septal stimulation on the intact kidney (relative to the denervated kidney) were similar to the effects of acute denervation and consistent with redistribution of intrarenal blood flow.

KEY WORDS

hypothalamus neural control of the kidney renal nerves
sympathoinhibition vasoconstriction pressor responses

Electrical stimulation of supramedullary neural structures has been known for many years to cause changes in blood pressure, heart rate, and organ blood flow; the patterns of response are related to the parameters of stimulation and the brain area stimulated (1-5). If integrative “centers” exist, they are apparently for specific physiological response patterns, e.g., exercise, defense reaction, or thermoregulation (5-8), rather than for isolated control of blood flow to specific organs (9-10). Consequently, it is not surprising that blood flow to any given organ, e.g., the kidney, may be affected by stimulation of several different areas of the brain, as has been shown for some species (4, 9-14). In the present studies, we stimulated various points in the septum and diencephalon of the rabbit to investigate areas of sympathoinhibitory and sympathoexcitatory control of the renal circulation. In addition, clearance techniques were used to define the nature of renal response.
Methods

Experiments were performed on 94 New Zealand rabbits anesthetized slowly with sodium pentobarbital, 30 mg/kg body weight. After completion of surgical preparation under an adequate level of anesthesia and prior to brain stimulation, the animals were immobilized with gallamine triethiodide (Flaxedil), 3 mg/kg, iv, and artificially ventilated with a Harvard respirator. No further anesthesia was given since the experiment lasted approximately 1 hour and often supplementary doses proved to be lethal. The weight (3-3.5 kg) and species of rabbit complied with the requirements of the atlas of stereotaxic coordinates by Sawyer et al. (15).

In all experiments the femoral artery and vein were cannulated, and arterial pressure was recorded continuously using a Statham P23AA pressure transducer and a Grass Polygraph. In some animals the heart rate was estimated from the blood pressure record at an accelerated chart speed. Bilateral retroperitoneal flank incisions were made to expose the kidneys and renal pedicles. When urine was collected, the ureters were cannulated bilaterally through a midline abdominal incision 2-3 cm long. Denervation of the left kidney was accomplished by freeing the pedicle of all connective tissue, severing all visible nerves, and painting the pedicle with xylocaine. Blood flow was measured with electromagnetic flow probes placed either on the renal arteries or on one renal artery (not denervated) and one femoral artery. The probes were connected to a dual-channel flowmeter (Biotronex BL-610), and the output was displayed on the same chart of the polygraph as was the blood pressure. All incisions were closed and the rabbit was turned onto its abdomen.

The head was fixed in a head holder designed for rabbits and used with a Baltimore Instrument Company stereotaxic apparatus. Skin flaps were retracted and a hole 3 mm in diameter was drilled at the appropriate site on the skull. For electrical stimulation and production of lesions a concentric, bipolar electrode, insulated except at the tip, was unilaterally and stereotaxically placed. The brain was perfused with 10% formalin, removed, and placed in formalin for further fixation. Frozen sections 40μ thick were mounted and stained with thionin. The exact position of the lesion was noted and other sites of stimulation were corrected accordingly.

When clearances were measured, the contents of the "priming" and sustaining solutions were as reported previously (16); the measurements of clearance, maximum tubular absorption of glucose (TmG), and maximum tubular secretion of PAH (TmPAH), and chemical analyses were done as described by Smith (17). Para-aminobiphrurate (PAH) clearance was used as an index of renal plasma flow and creatinine clearance as an estimate of glomerular filtration rate.

Urine from each kidney was collected before (10 minutes), during (10 minutes) and after (10 minutes) each test period, with 5 minutes allowed for dead-space washout between collections. Before and after the test periods, the electrode was in position but no stimulation was applied. Stimulation of the same site on alternate minutes began at the end of urine collection before the test period and 5 minutes prior to the test period and terminated at the end of the test period. A control urine collection was made prior to insertion of the electrode, and insertion per se was without consistent effect.

In some experiments, the effect of stimulation on urine formation was studied using a drop counter as an estimate that produced an electrical signal which was displayed simultaneously on the blood pressure and blood flow chart.

Results

CARDIOVASCULAR RESPONSES

Stimulation was applied to over 900 sites in the cortex, septum, and diencephalon anterior to the mammillary bodies. Some 600 of these were associated with changes in blood pressure exceeding 5 mm Hg. Pressor responses were more frequently (83%) obtained from the medial thalamic nuclei and most of the hypothalamus (Fig. 1). The mean evoked
A composite mapping of loci (triangles) in the areas of the diencephalon and septum which cause increases (left half of each serial section) and decreases (right half of each section) in blood pressure. Loci having no effect on blood pressure or renal blood flow are not represented. The upward or downward points of the triangles indicate increases or decreases, respectively, in the renal blood flow of the intact kidney. AC, anterior commissure; AHA, anterior hypothalamic area; AM, anteromedialis; AMYG, amygdala; DHA, dorsal hypothalamic area; DHM, dorsomedial hypothalamic area; FX, fornix; LHA, lateral hypothalamic area; LPO, lateral preoptic area; MM, medial mammillary; MPO, medial preoptic; MT, mamillothalamic tract; OCH, optic chiasma; PV, paraventricular area; RET, N. reticularis; SP, septum pellucidum; STH, subthalamus; VA, N. ventralis anterior; VM, centromedialis; VMH, ventromedial hypothalamic area (15).
The rises in systolic and diastolic pressure were 50 and 37 mm Hg, respectively. The maximum rises were 125 and 100 mm Hg. Depressor responses were more often obtained from two separate areas, the septum (57%) and ventral thalamic diffuse structures (52%). Stimulation in these two regions elicited similar falls in systolic and diastolic pressures: −18 and −16 mm Hg and −19 and −17 mm Hg, respectively. Typical examples of pressor and depressor responses illustrate the effects (Fig. 2). Mean latencies for blood pressure changes were 2 seconds regardless of the direction of change. Administration of atropine slightly augmented the pressor response and reduced the depressor response but the differences were small.

The pressor response was associated with progressive bradycardia (Fig. 2). In 31 observations the heart rate decreased from prestimulation control value of 218 ± 5 beats/min to peak response value of 171 ± 8 beats/min. Administration of atropine blocked the development of the bradycardia. The depressor response was not associated with a significant mean change in heart rate.

Some of the loci of stimulation evoking changes in renal blood flow are mapped in Figure 1. The cardiovascular pressor (left) and depressor (right) points of stimulation have been further subdivided into two groups: those points of stimulation evoking increased blood flow in the intact kidney (triangles) and those evoking a decrease (inverted triangles). Typical flow responses are shown in Figure 2.

Renal blood flow in the intact kidney decreased in 80% of the pressor responses and the mean change was −26 ± 1 (SE) ml/min (Fig. 3). Reduced renal blood flow was most often obtained by stimulation in the hypothalamic and medial thalamic areas. The mean latency of 2 seconds was comparable to that for blood pressure response. Simultaneously measured blood flow in the denervated kidney was invariably increased (+10 ± 1 ml/min). In the other 20% of pressor stimuli, renal blood flow in the intact kidney increased. This response occurred most frequently when stimuli were on the peripheral limits of the

**FIGURE 2**

Typical simultaneously measured changes in blood pressure and renal functions associated with stimulation of the hypothalamus, septum, and ventral thalamus. In each record from top down, the blood pressure (BP); intact kidney blood flow (I-RBF); denervated kidney blood flow (D-RBF); intact kidney urine flow (I-U); and denervated kidney urine flow (D-U), in drops. Stimulation was applied continuously during the period between the arrows.
pressor areas or in the areas more often evoking a depressor response. The increased flow (+18 ± 3 ml/min) was accompanied by a comparable or slightly greater increase in blood flow in the denervated kidney (+22 ± 4 ml/min). However, in most pressor responses, the blood flow tended to decrease in both kidneys after about 10 seconds of latency.

Femoral artery flow did not follow any predictable pattern of change, increasing in 29 of 62 stimuli evoking decreased renal blood flow and in 18 of 27 stimuli evoking increased renal blood flow. In the remainder, femoral artery flow decreased.

When central stimulation decreased blood pressure, blood flow in the intact kidney was decreased in 49 cases and increased in 39. The divergent effects on blood flow were associated with the two different depressor areas (Fig. 1). Stimulation in the ventral thalamic area usually (81%) decreased blood flow in the intact kidney (−8 ± 1 ml/min) in parallel with a fall in flow in the denervated kidney (−8 ± 1 ml/min). On the other hand, stimulation in the septal area usually (62%) increased blood flow in the intact kidney (+5 ± 1 ml/min) while decreasing blood flow in the denervated kidney (−5 ± 1 ml/min).

Femoral artery flow was estimated on only 17 occasions for both depressor groups and decreased in all but 3 of these.

After injection of atropine or during infusion of norepinephrine, both pressor and depressor stimuli changed blood flow in the intact kidney to about the same extent as they had before drug administration. Norepinephrine infusion appeared to attenuate the response of the denervated kidney to pressor stimulation.

CLEARANCE STUDIES

In five animals with both kidneys intact, unilateral brain stimulation was applied to test for crossover of fibers. During both pressor and depressor responses, regardless of side stimulated, changes in renal function were equally obtainable, and left and right kidneys responded identically. The ratio of left to right kidney function remained within the range of 1.0 ± 0.15 and did not significantly differ from unity for urine flow, PAH or creatinine clearances, or Na, K, or osmolar excretion rates. Therefore, the responses in this study were evaluated and based on unilateral stimulation.

Electrical stimulation of the hypothalamus which evoked a pressor response was accompanied by statistically significant reductions (P < 0.05) in all functions observed in the intact kidney. There were no significant changes in function in the denervated kidney (Table 1). The base-line renal blood flow and glomerular filtration rate were similar in both kidneys, however water and electrolyte excretion was higher on the denervated side. Changes in urine flow (using the drop counter as an index) occurred simultaneously with the changes in blood flow.

When ventral thalamic stimulation elicited a depressor response, the only significant changes that occurred were the bilaterally decreased sodium excretion rate (U_{NaV}) and fraction of filtered sodium excreted (C_{Na}/C_{GFR}).

Septal stimulation resulted in a fall in blood pressure comparable to that seen during ventral thalamic stimulation. However, sodium excretion in the intact kidney tended to
Changes in Renal Functions in the Denervated and Intact Kidney During Brain Stimulation

<table>
<thead>
<tr>
<th>Function</th>
<th>Hypothalamus (N = 13)</th>
<th>Septum (N = 6)</th>
<th>Ventral thalamus (N = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>I</td>
<td>D</td>
</tr>
<tr>
<td>CPAH</td>
<td>-0.4 ± 0.8</td>
<td>-4.4 ± 0.7*</td>
<td>-1.2 ± 0.4</td>
</tr>
<tr>
<td>Ccr</td>
<td>-0.12 ± 0.2</td>
<td>-1.8 ± 0.2*</td>
<td>-0.2 ± 0.2</td>
</tr>
<tr>
<td>FF</td>
<td>0.6 ± 1.0</td>
<td>-3.5 ± 0.6*</td>
<td>0.0 ± 0.9</td>
</tr>
<tr>
<td>V</td>
<td>-0.02 ± 0.02</td>
<td>-0.15 ± 0.02*</td>
<td>-0.04 ± 0.06</td>
</tr>
<tr>
<td>Csm</td>
<td>0.0 ± 0.04</td>
<td>-0.24 ± 0.03*</td>
<td>-0.03 ± 0.06</td>
</tr>
<tr>
<td>UNAV</td>
<td>-1.2 ± 4.7</td>
<td>-14.0 ± 5.1*</td>
<td>-4.8 ± 5.5</td>
</tr>
<tr>
<td>CUNAV/Ccr</td>
<td>-0.4 ± 0.5</td>
<td>-1.1 ± 0.4*</td>
<td>-1.0 ± 0.9</td>
</tr>
<tr>
<td>UKV</td>
<td>-0.0 ± 0.3</td>
<td>-1.7 ± 0.1*</td>
<td>0.0 ± 0.4</td>
</tr>
</tbody>
</table>

CPAH, Ccr, V, and Csm are ml/min; UNAV and UKV are μEq/min = ± SE; FF and CUNAV/Ccr are percent = ± SE.

*Statistically significant (P < 0.05) difference between average of pre- and postcontrol periods and stimulation period.
†Differ significantly from intact kidney during ventral thalamic stimulation.

Discussion

The cardiovascular and renal hemodynamic changes evoked by electrical stimulation and the distribution of points of stimulation in rabbits differ slightly from those in the dog and cat (12, 18-23). Pressor points appeared to be more frequently encountered throughout the hypothalamus, an area that is comparatively more extensive in rabbits, and depressor points were less often obtained in the hypothalamus. Hypothalamic pressor stimulation gradually decreased heart rate in these rabbits but increases heart rate in dogs and cats (22, 24, 25). In rabbits the net effect on heart rate seemed to depend on baroreceptor-evoked inhibition partially counteracted by accelerator fibers activated by hypothalamic stimulation. Evidence for this statement is threefold: administration of atropine blocked the bradycardia, the bradycardia did not correlate with the rise in blood pressure, and heart rate slowed even further immediately after termination of the brain stimulation. The depressor response evoked from the septum and ventral thalamus was not associated with a significant change in heart rate. Studies on rabbits (26, 27) have indicated interaction between reflex and suprabulbar effects which may result in alteration in the pressure "set point" around which the medullary control of heart rate and peripheral vascular resistance is most responsive.
Participation of renal vasoconstrictor fibers in these studies and previously by others (9-13, 27) have shown variable activation of sympathetic renal nerves during hypothalamic stimulation. Similarity in these rabbits, the extent of renal participation varied depending on the brain region stimulated. The pressor response was associated with changes in renal blood flow which ranged from a marked decrease to an increase equal to that seen in the denervated kidney.

**TABLE 2**

Effect of Brain Stimulation on $T_m_G$ and $T_m_{PAH}$ in the Intact Kidneys

<table>
<thead>
<tr>
<th>Function</th>
<th>Presor response</th>
<th>Depressor response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypothalamus ($n=5$)</td>
<td>Septum ($n=5$)</td>
</tr>
<tr>
<td></td>
<td>$E_1$</td>
<td>$E_2$</td>
</tr>
<tr>
<td>$C_r$</td>
<td>10.1 ± 1.5</td>
<td>4.7 ± 0.9*</td>
</tr>
<tr>
<td>$T_m_G$</td>
<td>40.9 ± 6.2</td>
<td>21.6 ± 4.6*</td>
</tr>
<tr>
<td>$T_m_{PAH}$</td>
<td>4.1 ± 0.6</td>
<td>3.0 ± 0.6</td>
</tr>
</tbody>
</table>

$C_r$ is in ml/min; $T_m_G$ and $T_m_{PAH}$ are in mg/min. The values are means ± se. $E_1$ and $E_2$ are pre- and poststimulation control periods with electrode in position, $S$ is stimulation period. $C_r = clearance$ of creatinine; $T_m_G = maximum tubular transport of glucose$; $T_m_{PAH} = maximum tubular transport of PAH$.

*Statistically significant ($P < 0.05$) difference between average of pre- and postcontrol periods and stimulation period.
Renal Response to Brain Stimulation

Atropine. This inhibition of renal vasoconstriction was found during the septal depressor response but not during the ventral thalamic depressor response. On the other hand, increased femoral blood flow was seen only during the ventral thalamic depressor response (3 of 15 observations with 3 showing no change), suggesting either a sparse central representation or location of inhibition of sympathetic control of the femoral vascular bed in other regions of the brain.

In brief, we have found that neurogenically mediated renal vasoconstriction may be elicited by stimulation in many parts of the hypothalamus of the rabbit and that inhibition of sympathetic renal vasoconstriction may be obtained by stimulation in the septal area. No discrete center for the control of renal blood flow could be demonstrated, but stimulation in the perifornical region of the hypothalamus most consistently decreased renal blood flow markedly. The renal responses to perifornical pressor and septal and ventral thalamic depressor responses have been investigated by clearance techniques.

Regardless of the limitations of these techniques and the protocol of stimulation, the evidence for neurally mediated vasoconstriction, decreased glomerular filtration, and water and electrolyte excretion in innervated kidneys during perifornical stimulation is clear. When both kidneys were intact, the changes were observed bilaterally during unilateral brain stimulation. When one kidney was denervated, that kidney failed to respond directly to brain stimulation and the differences between the two kidneys were statistically significant. Obviously, the renal nerves were essential for kidney participation, although humoral agents or autoregulation, or both, may have modified the response. The latter would, if anything, have minimized the differences between the innervated and denervated kidneys. These hemodynamic results agree with previous reports with respect to the relative role of the humoral component of the response. Wise and Ganong (13) found areas which, when stimulated, increased water and electrolyte excretion without changing blood flow or filtration rate. We found no such areas in the hypothalamus, and only sympathetic inhibition was seen in the septum.

The changes in functions in the intact kidney during the pressor response resembled those observed by many investigators during direct electrical stimulation of the renal nerves (30-32). The reductions in function were presumably based largely on complete halt to filtration in a fraction of the nephrons since $T_m$ and glomerular filtration rate were equally decreased. The filtered load offered to the nephrons during the stimulus remained higher than control $T_m$ in three of the five experiments and partial reduction of filtration in all nephrons could not have explained the reduction of $T_m$ and glomerular filtration rate to the same extent. Why $T_mPAH$ decreased less than $T_m$ is not clear unless partial shutdown (decreasing filtration but not secretion of PAH) or redistribution of filtration or both also occurred.

The denervated kidney did not show any changes in PAH or creatinine clearances, whereas direct blood flow measurements did show an increase during the pressor response. This discrepancy presumably related to the time of measurement, the direct measurements being for the initial minute of stimulation whereas clearance estimates were not begun until stimulation had been on for 5 minutes. Hence, the lack of delayed effect on renal plasma flow or glomerular filtration rate in the denervated kidney could presumably result from autoregulation or a humoral contribution, or both.

Septal depressor stimulation was associated with inhibition of existing neural tone as indicated by direct blood flow measurements. If the response persisted, a denervation type of diuresis (16) would be expected in the innervated kidney, i.e., an increase in urine flow, Na excretion, and $C_{Na}/C_{er}$ compared to the denervated kidney or the innervated kidney during ventral thalamic depressor stimulation. The changes seen were appropriate in direction and statistically significant when denervated-intact ratios for $C_{Na}/C_{er}$ were calculated for septal stimulation. Also, sodium...
excretion and $C_{Na}/C_{er}$ were significantly greater in the intact kidney during septal stimulation than during ventral thalamic stimulation. These comparisons suggest a persistent decrease in sympathetic tone during the septal depressor response. Ventral thalamic depressor stimulation, on the other hand, affected the intact and denervated kidneys in a similar manner and the effect was more likely secondary to a change in blood pressure (33, 34).

These studies provide no direct evidence for or against intrarenal redistribution of blood flow (16, 35). They do indicate the difficulty of demonstrating changes in electrolyte excretion independent of vascular effects when the sympathetic supply to the kidney is directly activated in the anesthetized animal (32). Part of the difficulty may be that pentobarbital anesthesia by itself activates those neurally mediated mechanisms for conserving salt that are independent of decreased total glomerular filtration rate (36, 37). Consequently, increased activation of the sympathetic nervous system at the supramedullary or peripheral nerve level (30, 32) will engage vasoconstrictor fibers in sufficient numbers to decrease renal blood flow, glomerular filtration, and sodium excretion. However, inhibition of this anesthesia-induced sympathetic activity can increase sodium excretion without changing glomerular filtration rate, as has been shown by septal stimulation in these experiments and by renal denervation in others (18); the mechanism might be cortical redistribution of flow.

References
RENAL RESPONSE TO BRAIN STIMULATION

20. Manning, J.W., Charbon, G.A., and Cotten, M.DeV.: Inhibition of tonic cardiac sympathet-

21. Folkow, B., Johansson, B., and Öberg, B.: Hypothalamic structure with a marked inhibi-

22. Bolme, P., Ngai, S.H., Uvnäs, B., and Wallenberg, L.R.: Circulatory and behavioral ef-
efects on electrical stimulation of the sympathetic vasodilator areas in the hypothalamus and

23. Manning, J.W., Jr., and Peiss, C.N.: Cardio-
vascular responses to electrical stimulation in
the diencephalon. Am J Physiol 198:366–370,
1960.

24. Djokosugito, A.M., Folkow, B., Kylstra, P.H.,
Lisander, B., and Tuttle, R.S.: Differentiat-
ed interaction between the hypothalamic de-
ference reaction and baroreceptor reflexes:
Effects on heart rate and regional flow resis-
tance. Acta Physiol Scand 78:376–385,
1970.

25. Tuttle, R.S., and McClean, M.: Central
inhibition of the defence reaction. Am J

26. Uther, J.B., Hunyor, S.N., Shaw, J., and
Kornrer, P.I.: Bulbar and suprabulbar control of
the cardiovascular autonomic effectors
during arterial hypoxia in the rabbit. Circ Res

27. Kornrer, P.I.: Central nervous control of au-
tonomic function: Possible implications in the
pathogenesis of hypertension. Circ Res 27

28. Ninomiya, I., Judy, W.V., and Wilson, M.F.: Hy-
pothalamic stimulus effects on sympathetic
1970.

medullary and adrenal cortical response to
stimulation of diencephalon. Am J Physiol

function during stimulation of renal

and function in separate kidneys during
stimulation of renal artery nerves in dogs. Am J

32. Blake, W.D.: Some effects of dihydrogenated
ergot alkaloids on renal hemodynamics, water
and electrolyte excretion in dog. Am J

33. Study, R.S., and Shipley, R.E.: Comparison of
direct with indirect renal blood flow, extraction
of inulin and diodrast, before and during acute
453, 1950.

34. Blake, W.D., Wegrin, R., Ward, H.P., and
Franke, C.W.: Effects of renal arterial constric-
tion on excretion of sodium and water. Am J

35. Pomerantz, B., Birtch, A.C., and Barger, A.C.: Neu-
ral control of intrarenal blood flow. Am J

ects of unilateral division of splanchic nerve on the renal excretion of electrolytes in
unanesthetized and anesthetized dogs: Mecha-
nism of “crossed stimulation.” Am J Physiol
175:363–374, 1953.

37. Blake, W.D.: Some effects of pentobarbital
anesthesia on renal hemodynamics, water and
electrolyte excretion in the dog. Am J Physiol
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