Role of Acetylcholine in the Renal Vasoconstrictor Response to Sympathetic Nerve Stimulation in the Dog

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ABSTRACT
The renal blood vessels were studied for a cholinergic determinant of their response to renal nerve stimulation. In dogs anesthetized with morphine and chloralose, control values were: mean aortic blood pressure 147 ± 4 mm Hg, renal blood flow 257 ± 12 ml/min. Acetylcholine (ACh) in 1-, 10- and 100-μg doses (all drugs given into the renal artery) increased renal blood flow, whereas 1000 μg of ACh reduced renal blood flow by 35%. After atropine and physostigmine, 1 μg of ACh did not alter renal blood flow, while 10, 100 and 1000 μg of ACh reduced renal blood flow by 6, 40 and 72%, respectively. After reserpine pretreatment, ACh in all doses increased renal blood flow (range of the means from 20 to 30%). The vasoconstrictor action of ACh was also blocked by phentolamine, guanethidine and hexamethonium. The renal vasoconstrictor response to renal nerve stimulation was blocked by guanethidine, but not by hexamethonium. These observations suggest that hexamethonium opposes ACh by barring its entry into the nerve terminals, whereas guanethidine blocks the intraneuronal release of norepinephrine by ACh. The vasoconstrictor response to nerve stimulation was reduced by atropine and augmented by physostigmine. Hemicholinium, which blocks the synthesis of ACh, attenuated the renal vasoconstriction produced by nerve stimulation. The results suggest that ACh may function in the canine kidney to liberate norepinephrine during activity of the sympathetic nerves.

ADDITIONAL KEY WORDS autonomic control of renal blood vessels autonomic blockade of the release of norepinephrine atropine hemicholinium and adrenergic neurotransmission physostigmine Burn and Rand’s hypothesis cholinergic mechanisms guanethidine hexamethonium

Acetylcholine (ACh) in the presence of atropine may produce an effect similar to that produced by stimulation of the sympathetic nerve supply in some organs (1). An essential role for ACh in the liberation of the sympathetic neurotransmitter during stimulation of the sympathetic nerves has been proposed by Burn and Rand (2). However, a cholinergic link in the sympathetic mechanism mediating constriction of blood vessels has been sought unsuccessfully (3-5). The renal vasculature has not been studied in this regard. In the present study, the renal blood vessels were examined for a cholinergic determinant of their vasoconstrictor response to sympathetic nerve stimulation. The renal

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blood vessels are very sensitive to adrenergic nervous activity, thus any modification of this activity should be readily noted (6-7).

The results of the present investigation are consistent with, but do not prove, the participation of a cholinergic mechanism in the constriction of the renal blood vessels produced by stimulation of the sympathetic nerves to the kidney. The present study shows that ACh probably constricts the renal blood vessels by releasing catecholamines; an anticholinesterase agent enhanced the renal vasoconstrictor response to nerve stimulation and the administration of hemicholinium (HC3), which may block the synthesis of ACh, reduced the renal vasoconstrictor response to stimulation of the renal nerves.

Methods

Seventy-two male mongrel dogs, 19 to 30 kg, were anesthetized with morphine sulfate (2 mg/kg, subcutaneously) and chloralose (70 mg/kg, iv). Four dogs received 2.5 mg/kg of reserpine (Serpasil) im, 24 hr before the experiment. On the day of the experiment, 4 hr before beginning the experimental observations, 1.0 mg/kg of reserpine was given iv. The lungs were ventilated by a Starling Ideal pump through a Y-shaped glass tube inserted in the trachea.

A Sanborn oscillograph was used to record left renal blood flow and mean aortic blood pressure. The latter was measured by a Statham transducer from a catheter inserted in a retrograde direction into the left femoral artery. In 22 experiments, the renal blood flow was measured by a rotameter which recorded the venous outflows of the kidney. This method is fully described in an earlier publication (8). Heparin (Panheparin) (2 to 3 mg/kg) was administered iv. While renal arterial flow was interrupted for 2 min, the renal vein was cannulated. The effluent was passed through a Shipley-Wilson rotameter (200 ml), which was placed below the renal vein. After passage through the rotameter, the blood emptied into a reservoir. The blood was then returned to the femoral vein of the animal by a Sigma motor pump that was automatically activated by a predetermined level of blood in the reservoir, 40 ml, which was maintained throughout the experiment. The entire recording system contained no more than 120 ml of blood, about 6% of the dog's blood volume. An equal volume of plasma expander (6% gelatin solution) was given at the start of the experiment.

In 50 experiments, the blood flow of the left kidney was measured by an electromagnetic flowmeter (Medicon, M-4001). To demonstrate that the renal blood flows, as measured by either recording system, were similar, the blood flow of a single kidney was measured simultaneously by each recording system in 3 experiments, the venous outflow by a rotameter, the arterial inflow by an electromagnetic flowmeter. Renal blood flow at rest or during renal nerve stimulation, measured simultaneously by the two systems, differed by less than 10%. After the renal artery was gently freed from its bed, the probe of the electromagnetic flowmeter (2.5 or 3.0 mm i.d.) was placed on the vessel close to its origin from the aorta. The electromagnetic flowmeter was calibrated by perfusing 0.9% saline through the excised renal arterial segment from a gravity flow system. In 2 experiments, the accuracy of the in vitro calibration of the electromagnetic flowmeter for each of its experimental settings was confirmed by simultaneous direct measurements of renal blood flow from a T tube inserted into the renal vein. Zero flow was established by briefly occluding the renal artery immediately distal to the flowmeter probe at the time of probe placement, several times during the experiment and at the end of the experiment.

The nerves to the left kidney were isolated in the area between the aorta and left renal artery, and placed in bipolar silver electrodes which were submerged in mineral oil. Stimulation (Grass S55 stimulator) of 1-msec duration were applied at various frequencies and at supramaximal voltage. A 25-gauge needle with rubber tubing attached to its end was inserted into the left renal artery near its junction with the aorta to give close intra-arterial injections of drugs. Thus, the effects of the drugs in small dosage were confined to the left kidney. Injections of the drugs were made in a volume not exceeding 1 ml of physiologic saline. The drugs used were: levaterenol bitartrate (1-norepinephrine; Levophed), acetylcholine chloride, hexamethonium and physostigmine bromides, atropine sulfate, guanethidine sulfate (Ismelin), phenolamine methanesulfonate (Regitine) and hemicholinium dibromide (HC3) [4,4'-Biphenacyl bis (β-hydroxyethyl-dimethyl-ammonium bromide)]. The doses of the drugs refer to the salts, except for levaterenol which is expressed as the base.

The results were expressed as the percent change of renal blood flow from the control renal blood flow. The use of close intra-arterial administration of drugs prevented or reduced systemic effects. Furthermore, the effect of drugs on the renal blood vessels occurred 20 to 30 sec before there were any effects on blood pres-
TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Mean aortic blood pressure (mm Hg ± SE)</th>
<th>Renal blood flow (ml/min ± SE)</th>
<th>Renal vascular resistance ± SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial values</td>
<td></td>
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<tr>
<td>Mean ± SE</td>
<td>147 ± 4</td>
<td>257 ± 12</td>
<td>601 ± 28</td>
</tr>
<tr>
<td>Final values</td>
<td>118 ± 5</td>
<td>191 ± 8</td>
<td>644 ± 34</td>
</tr>
</tbody>
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*Resistance was calculated by dividing arterial pressure in millimeters of mercury by mean flow in milliliters per minute and multiplying by 100.

ure. Accordingly, the changes in renal blood flow were recorded as the maximal change in renal blood flow expressed as percent of control occurring before alterations of systemic blood pressure, if such occurred. The results of 12 experiments in which atropine and physostigmine were used were statistically treated by an analysis of variance (9).

Results

In 68 dogs, the initial and final values for renal blood flow, renal vascular resistance, and mean aortic blood pressure are contained in Table 1. The tabulated renal blood flows of 4 to 5 ml/g of renal tissue per min indicate low resting renal sympathetic vasoconstrictor activity (10). Between the initial and final measurements 2 to 3 hr elapsed, during which drugs were given and the procedures detailed in methods were performed.

EFFECT OF ACh ON THE RENAL BLOOD VESSELS

ACh injected into the renal artery produced vasodilatation at low doses (1 μg and 10 μg), a variable effect at 100 μg (range, −9 to +21%) and vasoconstriction invariably at the 1000-μg dose (Figs. 1 and 2). After the

![Figure 1](image1.png)

**Figure 1**

Effect of acetylcholine on renal blood flow before and after atropine and physostigmine (1 to 2 mg/kg into the renal artery) and in the reserpine-pretreated dog. Ordinate = change in renal blood flow (perfusion pressure was unchanged) expressed as percent of control; abscissa = log dose of acetylcholine. Each point is a mean derived from the number of observations shown in parenthesis, representing 4 reserpine-pretreated dogs, 6 dogs receiving acetylcholine and 7 dogs given acetylcholine after atropine and physostigmine. Vertical lines = 1 SEM.

![Figure 2](image2.png)

**Figure 2**

Changes in renal blood flow (measured by a rotameter) produced by acetylcholine (ACh) before and after atropine and physostigmine. All drugs were administered into the renal artery at the arrows. Atropine and physostigmine were given 3 min before the right panel. BP = blood pressure; a 22-kg dog was used.

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close intra-arterial administration of atropine and physostigmine (1 to 2 mg), vasoconstriction was produced by ACh at all doses except the lowest (1 µg), which had no effect on renal blood flow (Fig. 1) (P < 0.01 for the difference in the response to ACh at all doses after physostigmine and atropine). Atropine alone was sufficient to convert the renal vasodilatation to vasoconstriction in response to intra-arterial administration of ACh, but physostigmine was required for fullest expression of the vasoconstrictor effect of ACh. Thus, in 2 atropine-treated dogs, reductions in renal blood flow of 20 and 58% occurred before, and 35 and 88% after, giving physostigmine in response to 100 and 1000 µg of ACh, respectively.

In 4 dogs, reserpine treatment resulted in a renal vasodilator response to all doses of ACh given into the renal artery (Fig. 1). The means for the control values of renal blood flow and aortic blood pressure in reserpine-treated dogs of 159 ± 16 ml/min and 96 ± 3 mm Hg, respectively, were not included in the calculation of the control values of Table 1. Reserpine treatment did not modify renal vascular reactivity to levarterenol.

**FIGURE 3**

Schematic representation of the sites of actions of guanethidine (G), hexamethonium (H) and phentolamine (P) at the renal vascular neuro-effector. The effect of ACh is blocked at the renal vascular receptor (R) by hexamethonium, at the cell membrane of the postganglionic nerve ending by hexamethonium and within the nerve by guanethidine. The sympathetic nerve impulse is prevented by guanethidine from liberating the neurotransmitter (NE, norepinephrine = levarterenol) from its storage sites.

**FIGURE 4**

Blockade by phentolamine of the renal vasoconstrictor effect of ACh given into the renal artery. Renal blood flow was measured by a rotameter. Physostigmine and atropine were given 5 min before; a 30-kg dog was used.
Hexamethonium (1 to 2 mg) and guanethidine (10 mg) administered into the renal artery blocked or reduced the renal vasoconstriction produced by close intra-arterial injections of ACh in the presence of atropine and physostigmine (1 to 2 mg given into the renal artery) presumably by preventing release of norepinephrine (12, 13) (Fig. 5) ($P<0.01$ for the changes produced by both hexamethonium and guanethidine to both doses of ACh). Thus, guanethidine abolished the renal vasoconstrictor effect of ACh in every instance, whereas hexamethonium blocked this action of ACh in 4 of 10 observations in 5 dogs. After hexamethonium, the mean reduction in renal blood flow produced by 1000 $\mu$g ACh intra-arterially was 27% (Fig. 5). When hexamethonium had incompletely blocked the renal vascular response to 1000 $\mu$g of ACh, subsequent administration of guanethidine (5 to 10 mg intra-arterially) eliminated the vasoconstrictor effect of ACh.

To separate their different actions at the postganglionic sympathetic nerve ending (Fig. 3), the effects of hexamethonium and guanethidine given intra-arterially were determined during the response of renal blood flow to nerve stimulation as well as to ACh given into the renal artery (Figs. 6 and 7). The renal vasoconstriction produced by renal nerve stimulation was not modified significantly by hexamethonium, whereas guanethidine significantly reduced ($P<0.01$ for all frequencies) the constriction produced by nerve stimulation (Figs. 6 and 7). In 3 experiments, atropine (1 mg intra-arterially)
did not change the effect of hexamethonium on the vasoconstrictor action of nerve stimulation. The lack of effect of hexamethonium on the response to renal nerve stimulation is to be contrasted with its attenuating the renal vasoconstrictor effects of injected ACh (Figs. 5 and 6).

After the administration of hexamethonium into the renal artery, the effect of ACh was abolished, whereas the vasoconstrictor response to nerve stimulation at frequencies of 5 and 10 cycle/sec was intact (Fig. 6). Administration of guanethidine intra-arterially then blocked the renal vasoconstrictor response to nerve stimulation.

**ALTERATION OF THE RENAL VASCULAR RESPONSE TO NERVE STIMULATION**

The response of renal blood flow to cycles of renal nerve stimulation of successively higher frequencies was reproducible, if rest periods alternating with periods of stimulation were observed, which minimized the development of a decremental response (Fig. 8). As a result of 5 pilot experiments, it was determined that the duration of nerve stimulation should not exceed 40 sec and succes-

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**Figure 7**

Effect of guanethidine (1 to 10 mg/kg) and hexamethonium (1 to 2 mg/kg) given into the renal artery on the renal vasoconstrictor response to renal nerve stimulation applied for 40 sec at the frequencies indicated on the abscissa. Each point is a mean derived from 6 observations (12 dogs, 4 in each group). Brackets represent ± SEM.

**Figure 8**

Response of renal blood flow (rotameter) to 2 sequences of nerve stimulation applied at the frequencies (cycle/sec) indicated at each arrow for 40 sec. The left and right panels are separated by 3 min. BP = blood pressure; a 23-kg dog was used.
sive stimuli should be separated by intervals of at least 1 min (Fig. 8). Adherence to these characteristics of stimulation resulted in predictable responses of renal blood flow to 2 or more successive cycles of nerve stimulation, if the frequency of nerve stimulation was 10 cycle/sec or less. It was necessary to distinguish spontaneous decremental responses (albeit small, given the aforementioned precautions) to 2 successive cycles of nerve stimulation, because the drugs used, atropine or physostigmine, may have produced similar changes. To determine the significance of drug intervention, 12 experiments (4 control, 4 atropine, and 4 physostigmine) were designed to determine statistical significance by an analysis of variance (9). The changes in renal blood flow were determined to 2 successive cycles of nerve stimulation at frequencies of 1, 2, 3, 5, 7 and 10 cycle/sec before and after giving either atropine or physostigmine (1 to 2 mg, intra-arterially) between the first and second cycles. The relationship between the control (without drug) responses of the renal blood vessels to 2 successive runs of nerve stimulation and the renal vasoconstrictor responses as modified by the administration of either atropine or physostigmine between the first and second cycles of renal nerve stimulation are depicted in Figure 9; arbitrary units represent the differences between the percent reduction in renal blood flow at the indicated frequencies for 2 successive cycles of nerve stimulation. For the data so obtained, each line in Figure 9 represents the average of 4 experiments. In the experiments in which neither atropine nor physostigmine was given, a slight decremental response was noted to occur spontaneously during the second cycle of nerve stimulation relative to the first (Fig. 9). The line above the zero line (physostigmine) in Figure 9 indicates an augmentation of the vasoconstrictor response during the second cycle of nerve stimulation relative to the first, physostigmine having been administered after the completion of the first cycle ($P < 0.01$ for the changes in the renal vascular response produced by physostigmine relative to the response of the untreated dogs). When atropine was administered between 2 cycles of nerve stimulation, a diminution in the renal vasoconstrictor response was observed after atropine, which differed significantly ($P < 0.01$) from the control or untreated response (Fig. 9).

The modification of the renal vasoconstric-

![Figure 9](image-url)
ator response to nerve stimulation might have been due to an effect of either drug at the adrenergic α-receptor, unrelated to their assumed primary action on cholinergic transmission. Thus, atropine was reported to reduce nonspecifically the effect of catecholamines at some neuro-effector sites (14), while physostigmine was shown to enhance the responses of the nictitating membrane to catecholamines (15). Levarterenol (1.0 μg/kg, intra-arterially) produced equivalent reductions of renal blood flow of 85% before, and 88% after, 2 mg of atropine. Similarly, 0.1 μg/kg of levarterenol administered intra-arterially produced equivalent reductions of renal blood flow of 52% before, and 64% after, 2 mg of atropine (all drugs administered into the renal artery). Under the present experimental conditions, physostigmine did not augment the renal vasoconstriction produced by levarterenol. A 63% reduction in renal blood flow occurred in response to 1.0 μg/kg of levarterenol. After 2 and 4 mg of physostigmine, 58% and 54% reductions of renal blood flow, respectively, were produced by levarterenol (all drugs administered into the renal artery). Under the present experimental conditions, physostigmine did not augment the renal vasoconstriction produced by levarterenol. A 63% reduction in renal blood flow occurred in response to 1.0 μg/kg of levarterenol. After 2 and 4 mg of physostigmine, 58% and 54% reductions of renal blood flow, respectively, were produced by levarterenol (all drugs administered into the renal artery). Under the present experimental conditions, physostigmine did not augment the renal vasoconstriction produced by levarterenol. A 63% reduction in renal blood flow occurred in response to 1.0 μg/kg of levarterenol. After 2 and 4 mg of physostigmine, 58% and 54% reductions of renal blood flow, respectively, were produced by levarterenol (all drugs administered into the renal artery). Under the present experimental conditions, physostigmine did not augment the renal vasoconstriction produced by levarterenol.

EFFECT OF HEMICHOLINIUM (HC₃) ON THE RENAL VASCULAR RESPONSE TO NERVE STIMULATION

HC₃ was reported to produce a failure of transmission in sympathetic nerves by impairing the synthesis of ACh (16); the adrenergic blocking action of HC₃ was reported to be most easily demonstrated at high frequencies of nerve stimulation (17). To demonstrate failure of transmission induced by HC₃ in the renal nerves, it was necessary to stimulate the renal nerves repetitively. A decremental renal vasoconstrictor response occurred in the absence of HC₃ in response to supramaximal repetitive stimulation of the renal nerves, if the frequency of nerve stimulation, intervals between trains and the duration of the trains of stimuli were not optimal, namely, frequency of 10 cycle/sec, pulse duration of 1 msec, applied for 20 sec every 30 to 40 sec.

The experiments in which HC₃ was used are plotted in Figures 10 and 11. The control response of renal blood flow for Figures 10 and 11 was derived from the mean change in renal blood flow to 5 successive trains of stimuli produced by repetitive stimulation of the renal nerve, obtained 10 min after beginning nerve stimulation. At the intervals indicated on the abscissa in Figures 10 and 11, the mean of the renal vasoconstrictor response was again determined to 5 successive trains of nerve stimulation and the mean values were expressed as percent change from the control vasoconstrictor response to renal nerve stimulation. In Figure 10, the mean of the renal vasoconstrictor responses to nerve stimulation of dogs not receiving HC₃ is indicated by the solid line. The same control experiments are plotted separately in Figure 11 to the left of zero time.

In Figure 10, HC₃ (0.5 to 2.0 mg/kg) was administered at zero time into the renal artery. At the arrows marked HC₃, supplementary HC₃ (0.5 mg) was administered. After 140 min of nerve stimulation, extinction of the renal vasoconstrictor response occurred following two supplementary doses in 1 experiment. To establish whether a significant change was produced by HC₃ on the renal vasoconstrictor response to nerve stimulation, the confidence limit (P = 0.05) for the control responses was determined to be 41% of control renal blood flow (18). Thus, in 2 of the 3 experiments of Figure 10, HC₃ significantly altered the response to renal nerve stimulation.

In Figure 11, the renal vasoconstrictor response to repetitive nerve stimulation was observed for 60 to 120 min before giving HC₃ and is plotted to the left of zero time. At zero time, HC₃ (1 to 2 mg/kg) was given into the renal artery in 4 experiments and the results obtained are plotted on the right of zero time. Choline has been reported to reverse the effects of HC₃ on nerve transmission (19). In 3 experiments, choline (9 mg) given into the renal artery augmented the
FIGURE 10
Effect of hemicholinium (0.5 to 2.0 mg/kg) given at zero time on the renal vasoconstrictor response to repetitive renal nerve stimulation (see text for variables of stimulation). Three experiments in which hemicholinium was used are indicated by the interrupted lines. The control response is indicated by the solid line and the number of dogs used to obtain each point is contained in parenthesis. Brackets represent ± SEM. Supplementary administration of HCS (0.5 mg into the renal artery) is indicated at the arrows. In 1 experiment, choline (9 mg) was given into the renal artery at the time indicated by the arrow.

FIGURE 11
In the left panel, the renal vasoconstrictor response to repetitive nerve stimulation (see text for variables of stimulation) of 60 to 120 min is indicated for 6 experiments. At zero time, hemicholinium (1 mg/kg) was given into the renal artery in 4 of the 6 experiments and changes in the vasoconstrictor response were followed for 60 to 80 min. At the arrows either additional HCS (0.5 mg) or choline (9 mg) was given. Heavy lines indicate effect of choline.

FIGURE 12
Renal vasoconstrictor response to nerve stimulation applied at the bars (same variables of stimulation). Renal blood flow was measured by an electromagnetic flowmeter. Hemicholinium was given into the renal artery 60 min before the middle panel and choline 5 min before the panel on the right. BP = blood pressure; a 17-kg dog was used.
renal vasoconstrictor response which was proceeding to extinction (Figs. 10 through 12).

In Figure 12, HC₃ was given after 2 hr of an undiminished renal vasoconstrictor response to repetitive renal nerve stimulation. The middle panel of the trace was obtained after giving HC₅. Choline partially restored the vasoconstrictor response to renal nerve stimulation.

The reactivity of the renal blood vessels to levarterenol and ACh was unchanged after giving HC₂. In 4 experiments, levarterenol (0.05, 0.1, 1.0 μg/kg) and ACh (10 and 100 μg total dose), given intra-arterially, had undiminished effects on renal blood flow after administration of HC₃. Thus, decreased reactivity of the autonomic receptors to these neurotransmitters was not a factor in the reduced renal vasoconstrictor response produced by HC₂.

**Discussion**

Several lines of evidence provided by the present data suggest a role for ACh in the release of the sympathetic neurotransmitter in response to the sympathetic nerve impulse: (1) ACh, which mimicked the effects of renal nerve stimulation, produced renal vasoconstriction by releasing catecholamines; (2) the administration of an anticholinesterase agent (physostigmine) augmented the vascular response to renal nerve stimulation; (3) the constriction of the renal blood vessels produced by stimulation of the renal nerves was attenuated by HC₅ which blocks the synthesis of ACh. An expansion of the major observations follows.

Though the renal vasodilator effect of ACh is well known (20-22), constriction of the renal blood vessels produced by ACh has received scant attention (23). Under the present experimental conditions, 1 mg of ACh administered into the renal artery produced vasoconstriction (Figs. 1 and 2). In the presence of atropine and physostigmine, renal vasoconstriction produced by ACh was augmented and its threshold was considerably lowered (Figs. 1 and 2). After reserpine, ACh caused only renal vasodilatation (Fig. 1).

The vasoconstrictor effect of ACh was probably produced by release of catecholamines, presumably from adrenergic nerve terminals, since it was readily blocked by autonomic blocking agents. Four autonomic blocking agents, each with different sites of activity at the neuro-effector, e.g., pre- or postjunctionally, were used to block the vasoconstrictor effect of ACh (Figs. 1, 3 and 4). These were: (1) reserpine-pretreatment, which depletes catecholamines (Fig. 1); (2) phentolamine, which blocks the adrenergic α-receptors that mediate constriction of the renal blood vessels produced by catecholamines released from renal sympathetic nerve endings by ACh (Fig. 4); (3) guanethidine, which prevents the renal vasoconstrictor effect of ACh prejunctionally and so blocks the release of catecholamines by ACh from the sympathetic nerve endings (Figs. 3 and 5); and (4) hexamethonium which probably interferes with the action of ACh at the cell membrane of the adrenergic nerve ending or prevents its entrance into the nerve fiber. Hexamethonium, being a bis-quaternary compound, does not readily penetrate the nerve fiber (12), in contradistinction to guanethidine which gains entry into the nerve ending (24). This interpretation was strengthened by the observation in the present experiments that hexamethonium failed to block the renal vasoconstrictor response to renal nerve stimulation, whereas guanethidine blocked the renal vasoconstriction produced by ACh and nerve stimulation (Figs. 5 through 7). Hexamethonium was demonstrated by Blakeley, Brown and Ferry to oppose the release of catecholamines from sympathetic nerve terminals in the spleen produced by ACh, while failing to reduce the output of norepinephrine in response to splenic nerve stimulation (25).

The question of the presence and significance of intrarenal ganglia and nerve cells requires some qualification. Mitchell, as well as DeMuylder, was unable to find nerve cells within the mammalian kidney or considered them scarce (26, 27). Shvalev, though failing to note large numbers of renal nerve cells, observed a consistent localization of ganglia,
subcapsularly in the canine kidney (28). The present results are consistent with the absence or paucity of nerve cells related to adrenergic nervous vasoconstrictor activity, but do not exclude intrarenal ganglia related to other neurally dependent effects within the kidney.

A cholinergic mechanism in sympathetic nerve transmission was also suggested by the results derived from histochemical methods for detection of acetylcholinesterase. The presence within some adrenergic neurons of an intermediate concentration of acetylcholinesterase suggested to Koelle a cholinergic mechanism for those adrenergic nerves (29). The walls of the blood vessels of the canine kidney, in which most of the sympathetic nerves terminate, were described to stain markedly for cholinesterase (30). However, these observations do not exclude the possibility of a mixed and contiguous population of cholinergic and adrenergic nerves within the kidney.

The observations of Aström, Crafoord and Samelius-Broberg that ACh in high doses had a direct renal vasoconstrictor effect in the cat which was not blocked by adrenergic blocking agents may be explained by species difference (23). Thus, the nature of autonomic transmission for the nictitating membrane in the rabbit differs from the cat, for in the rabbit the effect of HC₃ suggests a cholinergic determinant of the adrenergic innervation of the nictitating membrane which was not present in the cat (31). Furthermore, the distribution and concentration of acetylcholinesterase-staining fibers in the adrenergic nerve supply of the nictitating membrane of the rabbit, but not the cat, demonstrated cholinesterase readily in renal blood vessels and nerves (30).

Physostigmine was reported to increase the response to postganglionic nerve stimulation at several neuro-effector sites (33, 34). This effect of physostigmine was interpreted by Burn and Weetman to be due to an increase in the ACh available for releasing catecholamines in response to sympathetic nerve stimulation (34). In the present study, previous renal intra-arterial administration of physostigmine enhanced the response to stimulation of the renal nerves (Fig. 9). Furthermore, this effect was not related to augmentation of the response to catecholamines, for the constriction of the renal blood vessels produced by intra-arterial injection of levarterenol was not increased.

Hyoscine, an atropine analogue, was reported by Burn, Dromey and Large to increase the response to stimulation of the sympathetic nerves to the rabbit ileum (35). According to their formulation, atropine should have enhanced the effect of sympathetic nerve stimulation, particularly at low frequencies, by preventing the vasodilator action of ACh on the renal blood vessels, thereby unmasking the fully developed vasoconstrictor response, such as occurred in response to injected ACh (Figs. 1 and 2). However, the present study demonstrated a reduction by atropine of the renal vasoconstriction produced by nerve stimulation (Fig. 9). A reduction by atropine of vasoconstriction produced by nerve stimulation was described by Bevan and Su (3). Atropine was suggested by Green and Carlini to have a prejunctional effect upon ACh release, in addition to blockade of cholinergic receptors (36). In the present work, a direct depression of vascular smooth muscle by atropine was considered unlikely, because levarterenol had an undiminished renal vasoconstrictor effect after atropine was administered.

HC₃ was demonstrated to reduce the response to nerve stimulation at neuro-effector sites for several species (17, 31). The primary effect of HC₃ on nerve transmission is interference with the synthesis of ACh, probably by competing with choline for carriers transporting choline to intraneuronal sites for acetylation (16). Repetitive nerve stimulation was required for interference with nerve transmission by HC₃, because a critical reduction of ACh cannot be effected by HC₃ alone in the face of low resting sympathetic nervous.
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To avoid progressive diminution of the renal vasconstrictor response to nerve stimulation in the absence of HC₃, it was necessary to observe strict (and perhaps weighted) criteria for the characteristics of nerve stimulation.

HC₃ was considered to diminish renal vasoconstriction in six of seven instances during nerve stimulation under the present experimental conditions (Figs. 10 and 11). This interpretation was strengthened by the following observations: (1) a nondecremental response of the renal blood vessels to nerve stimulation was noted for as long as 2 hr when HC₃ was not used (Figs. 10 and 11); (2) the effect of HC₃ was apparently dose-related, for the smallest amount was given in the experiment in which no attenuation of renal vasoconstriction occurred (Fig. 10) and supplementary HC₅ hastened the onset of the diminished response or reversed the appearance of an increased vasconstrictor response to renal nerve stimulation; (3) choline restored partially the attenuated vasconstrictor effect of renal nerve stimulation which occurred after giving HC₃ (Figs. 10 through 12). Inasmuch as inhibition of the transport of choline is probably responsible for the effects of HC₃ on ACh synthesis, the partial restoration by choline of the renal vasconstrictor response to nerve stimulation is presumably due to increased choline transport intraneuronally and subsequent acetylation of choline forming ACh.

Finally, the results of the present investigation permit only a tentative identification of the renal vascular segment(s) producing the increased vascular resistance in response to ACh or nerve stimulation. Stimulation of the renal nerves may produce a constriction of all arterial elements, large and small, whereas constriction of the renal venous elements is minimal (37). ACh, having released catecholamines, probably has a greater effect on the smaller renal resistance vessels, e.g., afferent arterioles as well as the venules. The basis for this tentative distinction derives from (1) studies which show differences in the effects on segmental resistances of the kidney between administered sympathomimetic agents and stimulation of the renal nerves and (2) the localization within the renal vasculature of tritiated norepinephrine.

Thus, stimulation of the renal nerves was reported to produce a constriction of all the renal arterial elements (38, 39), whereas administered sympathomimetic agents had their largest effect on the smaller renal vascular elements (38, 39), particularly those which are pregglomerular (40). The largest concentration of tritiated norepinephrine, which is presumably susceptible to release by ACh, was noted by Marks, Samorajski, and Webster (41) to be located at the vascular pole of the glomerulus.

Our results suggest that there is cholinergic participation in the response of the canine renal blood vessels to sympathetic nerve stimulation. Others have proposed that the sympathomimetic effect of injected ACh may be due to the setting up by ACh of antidromic impulses in postganglionic sympathetic nerve fibers, rather than an effect of ACh unrelated to initiation of nervous impulses (25, 42). Though an antidromic effect of ACh may explain the renal vasoconstriction produced by injected ACh, it does not account for the effects of phystostigmine and HC₃ on adrenergic renal vasoconstriction under the present experimental conditions. Lest the present paper be considered an unqualified endorsement of such a cholinergic link, it may well be that the postulated cholinergic mechanism is not essential for renal nerve transmission, but rather facilitative, such that it requires the highly artificial conditions of the laboratory for its expression.

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