Renal Vascular Response to Ganglionic Stimulants in the Dog

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Although the kidney is innervated with abundant autonomic nerves, the presence of ganglion cells in the kidney does not seem to have been morphologically demonstrated. However, pharmacological studies made by Page and McCubbin in the dog, and by Åström et al. in the cat suggested the existence of ganglion cells in the kidney. Presently renal vascular response to various ganglionic drugs and to renal nerve stimulation were studied in order to obtain further information on the mechanism and the site of ganglionic drugs in the dog's kidney.

Materials and Methods

Dogs weighing from 10 to 15 kg of either sex were anesthetized with 30-35 mg/kg pentobarbital sodium, given i.v., with small additional dose when needed for the maintenance of anesthesia. A glass T-tube was intubated into the trachea and a cannula was inserted into the left carotid artery. The left kidney was retroperitoneally approached through a left flank incision and the abdominal aorta was ligated about 6 cm below the bifurcation of the left renal artery. Heparin was injected intravenously in an initial dose of 300 U/kg, followed by 100 U/kg every 1-2 hours. A glass cannula devised by Gilmore connected with the left carotid artery by silico- nized polyethylene tubing was inserted into the abdominal aorta about 5 cm below the left renal artery and wedged into the left renal artery under a transient interruption (10-30 sec) of the aortic flow. The cannula was fit tightly in the artery so that no ligature was necessary to hold it in the place, and the blood was perfused by a sigmamotor pump which was placed in the circuit. An electromagnetic flow meter (Nikon Koden, Type MF-II) and a pressure transducer (Nikon Koden, Type MP IVT) were placed in the perfusion circuit in order to determine renal blood flow and renal perfusion pressure. Simultaneously, systemic blood pressure was recorded from the right brachial artery by an electric manometer (Nikon Koden, Type MP IVT). Drugs dissolved in 0.1 to 0.2 ml 0.9 percent saline solution were injected into the left renal artery or into the right brachial vein.

A series of experiments was performed in a cross-circulation preparation, preparing of which had been reported by the present authors. In the preparation vascular isolation and nervous connection between the recipient's kidney and its body were fully maintained.

In another experimental group, a heart-lung-kidney preparation was employed. In this preparation, the heparinized blood obtained from other dogs was perfused to the kidney with a sigmamotor pump at a constant flow rate. The perfusing blood was oxygenated or deoxygenated with a disk oxygenator, into which gas mixtures of O₂, N₂ or CO₂ in various concentrations were inflowed. The venous blood was returned to a blood reservoir. Blood samples for measuring Pco₂, Pco₂ and pH were taken from tubing which connects the renal arterial cannula. Analysis was done with IL meter (Industrial Laboratory) as previously described.

In some experiments, the renal nerve running along the renal artery or the splanchic nerve diverged from the thoracic sympathetic trunk was exposed for electrical stimulation. Square-wave impulses were delivered for 10 sec using MSE-3 stimulator (Nikon Koden). The parameters were: voltage, 2-20V; duration, 1 msec; frequency, 3-20 cps. In
an experimental group, renal nerves were cut 3 to 14
days before the experiments or conductivity was
blocked acutely with lidocaine. Several dogs
were injected with reserpine (0.5 mg/kg) subcuteously
for two successive days before experiment. Reserpinized
dogs were anesthetized with pento-
barbital sodium in a dose of 20 mg/kg. Renal vasculo-
ar resistance (RVR) was calculated from mean renal
perfusion pressure and renal blood flow.

The drugs used were:

Norepinephrine hydrochloride (Sankyo Seiyaku
K.K.), Epinephrine hydrochloride (Sankyo Seiyaku
K.K.), Tyramine hydrochloride (Sigma Chem. Co.),
1,1-Dimethyl-4-phenylpiperazinium iodide (DMPP)
(Park, Davis & Co.), Nicotine (Tokyo Kaseikogyo
K.K.), Acetylcholine chloride (Daichii Seiyaku K.K.),
Atropine sulfate (Fujisawa Yakuhinkogyo K.K.),
Phenoxybenzamine hydrochloride (Tokyo Kasei-
kogyo K.K.), 4-(2-Isopropylamino-1-hydroxyethyl)
methane sulfonilide hydrochloride (MJ 1999) (Mead
Johnson Research Center), Hexamethonium bromide
(C6) (Yamanouchi Seiyaku K.K.), Pentolinium
tartrate (C5) (Dainippon Seiyaku K.K.), Lidocaine
(Xylocaine) (Fujisawa Yakuhinkogyo K.K.), Reser-
pine (Dainippon Seiyaku K.K.), Brevetoxin tosylate
(Chugai Seiyaku K.K.), Cocaine (Takeda Seiyaku
K.K.), d-Tubocurarine chloride (Yoshitomi Seiyaku
K.K.), Heparin (Taiyo Gyogyo K.K.) and Pento-
barbital sodium (Dainippon Seiyaku K.K.).

RESULTS

A. Experiments with the kidney perfused
with the dog's auto-blood.

a) Experiments in normal dogs.

The dog's renal blood flow (RBF) fluctuated
at the beginning of perfusion, leading to a stable
state within a few minutes. The average RBF
and RVR in 76 dogs were 2.2 ml/min/g kidney
weight ranging from 1.2 to 3.3 ml/min/g and
1.21 mmHg/ml/min ranging from 0.75 to
2.2 mmHg/ml/min respectively. After the kidney was perfused with a sigmoidum pump
at a same flow rate as just before perfusion
RVR rapidly rose 10-15 per cent, and re-
mained at this level for the following 1-2
hours. The initial perfusion pressure was set
at 10-20 mmHg below the initial systemic
blood pressure.

1. Effect of acetylcholine.

The vasodilator effect of acetylcholine in-
jected into the renal artery (i.r.a.) depends
upon the initial perfusion pressure and a dose
given. When initial perfusion pressure was as
high as 40 mmHg or above, a dose as small as
0.5-1.0 μg produced vasodilatation, and a de-
crease in RVR became marked with an increase
in dosage up to 5.0 μg. Doses more than 5.0
μg caused a reduction of RVR associated with
a decrease in systemic blood pressure. Larger
than 500 μg produced a biphasic response in
RVR, a temporary increase and a successive
significant decrease, and a concomitant decrease
in systemic blood pressure for about 20 min.
Two to 5 mg of atropine (i.r.a.) caused a marked
but transient reduction in renal perfusion pres-
sure. After the perfusion pressure subjected to
atropine regained a normal level, no renal
vasodilator and systemic depressor response to
small doses of acetylcholine appeared. Here,
interestingly enough there appeared a modified
biphasic response of renal vessels to large doses
of acetylcholine. That is, the initial increase
in RVR was not lessened, but rather strength-
ened, and the second response lowering RVR
was decreased or sometimes reversed to ele-
vating RVR. In reversed cases, the first rise
in RVR was greater in its magnitude, but less
in duration than the second rise which was
usually accompanied by a slight systemic pres-
sor response (Fig. 1).

2. Effect of nicotine and DMPP.

Nicotine and DMPP, when given into the
renal artery in amounts much smaller than
those required to rise systemic blood pressure,
elicited a biphasic response in renal perfusion
pressure as with large doses of acetylcholine in
the presence of atropine. The first rise in perfu-
sion pressure appeared immediately after
injection and the second one 10-20 sec later
(Fig. 1.). In the first rise in perfusion pressure,
nicotine, on weight basis, was two times as
active as DMPP.

3. Effect of ganglionic blocking agents.

C6 (10 mg, i.r.a.) or C5 (3-5 mg, i.r.a.) caused
a marked decrease in systemic blood pressure
and renal perfusion pressure, followed by a
gradual return of both to a normal level within
30 min. The magnitude of these responses was
greater in dogs with high systemic blood pres-
sure than those with low pressure. The pretreat-
ment with the above two blocking agents did
not affect the vasodilator action of acetylcholine,
but blocked the biphasic increase in perfusion

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Fig. 1. Effect of acetylcholine and DMPP injected into the renal artery before and after atropine on the renal circulation.

RBF: Renal Blood Flow.
PP: Renal Perfusion Pressure.
BP: Systemic Blood Pressure.
Resp: Respiration.

d-tubocurarine (1–3 mg, i.r.a.) or of lidocaine (10–20 mg, i.r.a.) was also antagonistic to ganglionic stimulants, to less degree and with shorter duration than C6.

4. Effect of phenoxybenzamine.
Phenoxybenzamine (10–40 mg, i.r.a.) reduced renal perfusion pressure and blocked the biphasic response resulting from nicotine, DMPP and acetylcholine for about 2 hours. Needless to say, norepinephrine showed no effect upon systemic blood pressure and renal perfusion pressure (Fig. 3).

5. Effect of bretylium.
The renal vasoconstrictor response to DMPP (5–10 mg, i.r.a.) was depressed by 50–80 per cent within 5 min after intravenous injection of bretylium (5–7 mg/kg) and further depressed until 60 min after the injection. Even 2 hours later no complete recovery observed. While the inhibition against vasoconstriction induced by tyramine in doses above 50 µg was marked at the early stage after injection of bretylium, followed by a gradual recovery. Effect of exogenous norepinephrine was not blocked by bretylium but rather gradually potentiated.


Fig. 2. Effect of autonomic drugs and electrical stimulation of the renal nerve after hexamethonium on the renal circulation.

RBF: Renal Blood Flow.
PP: Renal Perfusion Pressure.
BP: Systemic Blood Pressure.
UF: Urine Flow.

pressure induced by nicotine, DMPP and acetylcholine in large amounts for a period of about 30 min. (Fig. 2). Renal arterial injection of

Tyramine (10–50 μg, i.r.a.) produced a transient increase, sometimes after a brief decrease, in perfusion pressure and a dose of more than 100 μg caused a considerably persistent increase. One hundred μg of tyramine was equipotent to 0.2 μg of norepinephrine in its vasoconstrictor action. Repeated injection of tyramine in a dose of 100–200 μg 8–10 times resulted in no tachyphylaxis in the renal vasoconstrictor action (Fig. 4).

7. Effect of cocaine.

During slow injection of cocaine in a dose of 5 mg (i.r.a.) there developed a fluctuation of perfusion pressure. From 5 min to over 2 hours after cocaine was administered tyramine (200–300 μg, i.r.a.) produced no significant action on renal perfusion pressure. Unlike tyramine, the vasoconstrictor action of DMPP was slightly suppressed at the early stage after administration of cocaine and recovered completely within 30 min (Fig. 5).

b) Experiments in adrenalectomized dogs.

It has been reported that ganglionic stimulants promote a catecholamine secretion from the adrenal gland and that the adrenal gland is humorally connected with the kidney through a fine network of arteries. Thereupon, effects of ganglionic stimulants on the renal vasculature were studied in adrenalectomized dogs. The right adrenal gland was removed surgically through an abdominal midline incision, and a single ligature was passed around a band of tissue connecting the left adrenal gland and the left kidney and tied tightly. In adrenalectomized dogs, renal arterial injection of ganglionic stimulants caused a monophasic elevation of perfusion pressure immediately after drugs without any changes in systemic blood pressure (Fig. 6). This monophasic response was completely blocked by Cl, as well as phenoxybenzamine as mentioned above (Fig. 2, Fig. 3).

c) Experiments in reserpinized dogs.

In reserpinized dogs systemic blood pressure and renal vascular resistance were averaged 70 mmHg and 0.9 mmHg/ml/min respectively and tyramine no longer had a vasoconstrictor action on the kidney, but responses to cate-
Fig. 4. Effect of tyramine injected into the renal artery on the renal circulation.

BP: Systemic Blood Pressure.  RESP: Respiration.

Fig. 5. Effect of DMPP and tyramine before and after cocaine on the renal circulation.


cholamines were potentiated as seen in other organs. DMPP (20–50 μg, i.r.a.) produced a slight elevation in both systemic and renal perfusion pressure without the initial elevation characteristically observed in unreserpinized dogs. This slight elevation was eliminated following adrenalectomy. Intravenous infusion of norepinephrine (0.1–0.5 μg/kg/min for 20 min) to reserpinized dogs restored responses to both drugs in various degrees.

d) Experiments in dogs with denervated kidney.

Left renal nerves were cut 3–14 days before experiments. Three days after denervation the renal vasoconstrictor action of DMPP and acetylcholine was observed as in the untreated kidney, whereas the action of tyramine was significantly intensified. On the seventh day after denervation the initial response to DMPP was partially inhibited and the response to tyramine diminished greatly. Ten to fourteen days after denervation, both tyramine and DMPP lost their immediate vasoconstrictor action completely and DMPP in large doses caused a slight vasodilatation, which could not be blocked by atropine (5 mg, i.r.a.) or MJ 1999 (5–10 mg, i.r.a.).

e) Experiments on renal and splanchnic nerve stimulation.

1. Stimulation of the renal nerve.

Electrical stimulation of the renal nerve (3–5 cps, 1 msec, 2–20 V, for 10 sec) produced a monophasic increase in renal perfusion pressure without any change in systemic blood pressure. The increase in renal perfusion pressure was perfectly suppressed by phenoxybenzamine (10–40 mg, i.r.a.), imperfectly by bretylium (5–7 mg/kg, i.v.) but not by C₆ (10–20 mg, i.r.a.) or lidocaine (10–20 mg, i.r.a.).

2. Stimulation of the splanchnic nerve.

Electrical stimulation of the splanchnic nerve (5–20 cps, 1 msec, 5–20 V, for 10 sec) caused a monophasic to a biphasic elevation in renal perfusion and systemic blood pressure with increasing frequency of stimuli. These responses were obviously blocked by phenoxy-
benzamine (2 mg/kg, i.v. or 10–40 mg, i.r.a.) or by C₆ (1 mg/kg, i.v. or 10 mg, i.r.a.) as described by Sakai³ (Fig. 7).

B. Experiments with a cross-circulation preparation.

In order to get a better insight into the site of the blocking action of C₆ a study was made using a cross-circulation preparation. Splanchnic nerve stimulation in the recipient dog under the conditions described above produced only a monophasic elevation in renal perfusion pressure. C₆ in a dose of 10 mg i.r.a. failed to block the effect of splanchnic nerve stimulation, but in a dose of 1 mg/kg i.v. to the recipient dog blocked it. These data indicate that the site of blocking action of C₆ is located at the extrarenal synapse.


It is generally believed that the nervous function, especially synaptic transmission, is easily disturbed by anoxia. Therefore, renal vascular responses to various drugs were studied using a heart-lung-kidney preparation perfused with the hypoxic blood. The method was previously reported in detail⁴. When the kidney was perfused with the oxygenated blood, its vascular responses to autonomic drugs, i.e., norepinephrine, isoproterenol, acetylcholine, tyramine, nicotine, DMPP and C₆ and to renal nerve stimulation agreed with those obtained in the kidney in situ described above. For example, C₆ was unable to block the constrictor action of renal nerve stimulation, but blocked that of nicotine, DMPP and large dose of acetylcholine. On the other hand, perfusion of the kidney with the hypoxic blood resulted in a gradual reduction in RVR with decreasing Pₐ O₂. When Pₐ O₂ decreased to 60–70 mmHg, RVR reduced by about 25 percent of the control. Under this condition, the vasoconstrictor
effect of norepinephrine and of renal nerve stimulation remained unchanged or slightly decreased, but that of tyramine was not modified or rather intensified. Unlike these results, the vasoconstrictor action of ganglionic stimulants was greatly reduced. Following oxygenation of the perfused blood, these depressed responses recovered in varying degrees depending upon the kind of drug.

**Discussion**

Ganglionic stimulants, nicotine, DMPP and acetylcholine in the presence of atropine, given into the renal artery produced a biphasic elevation in renal perfusion pressure. The first elevation was clearly blocked by phenoxybenzamine but also by ganglionic blockades and high doses of d-tubocurarine and lidocaine, but not by adrenalectomy. The second elevation and a concomitant rise in the systemic blood pressure were also abolished by phenoxybenzamine, C₆ and by adrenalectomy. These findings were qualitatively similar to those described by Page and McCubbin in the dog and by Åström et al. in the cat. On the other hand, catecholamine and tyramine produced a marked monophasic increase in renal vascular resistance, which was blocked by phenoxybenzamine, but not by ganglionic blockades. Either reserpiniization or renal nerve section in dogs resulted in a complete loss of the tyramine effect and of the first elevation induced by ganglionic stimulants. Furthermore, reserpinization caused a great reduction in the second elevation, which, however, needed further adrenalectomy for complete abolishment. These findings indicate that the first and the second rises in perfusion pressure induced by ganglionic stimulants were brought about differently, probably the former being originated in the kidney itself and the latter in the adrenal gland and that the both responses, however, were mediated by catecholamines liberated from the aforesaid organs. A belief that the presence of endogenous catecholamines is essential for the vasoconstrictor response to ganglionic stimulants is further supported by the finding that the responsiveness to tyramine and ganglionic stimulants in reserpinized dogs is easily restored by an intravenous infusion of norepinephrine.

Both cocaine and bretylium were also able to block the renal vasoconstrictor action of tyramine and ganglionic stimulants, but differ each other in their blocking pattern. Furthermore, it was observed that renal vessels decreased their response to ganglionic stimulants following perfusion of the kidney with the hypoxic blood, but showed a slight decline of response to norepinephrine and renal nerve stimulation, becoming more sensitive to the vasoconstrictor action of tyramine. It is well known that nervous functions, especially at the synaptic transmission, are disturbed by anoxia. These data strongly suggested that the site in the kidney, from which catecholamine is liberated by ganglionic stimulants, is not the commonly assumed storage site such as mobile pool I for which tyramine is responsible and that the catecholamine releasing mechanism of ganglionic stimulants differs from that of tyramine. Lee and Shideman demonstrated that ganglionic stimulants produce a transitory positive inotropic effect in the presence of atropine on the isolated papillary muscle of cats and that this effect was blocked by ganglionic blockades and dichloroisoproterenol. Futhermore, they showed the response thus developed was inhibited or markedly reduced following the administration of cocaine or ephedrine, or reserpine respectively, the latter administration being accompanied by a marked reduction in catecholamine content in the muscle. But they failed to reveal the presence of ganglion cells in the papillary muscle morphologically. Similar result were obtained by Jarrett in the ileo-sphincter of rabbits. He postulated a certain unidentified structure in this tissue, to which ganglionic stimulants may acts directly in liberating catecholamines. The present data were in good agreement with these reports, suggesting the existence of ganglion-like elements in dog's renal vessels, even in the absence of anatomical evidence.

As generally accepted the stimulation of the renal nerve produced a renal vasoconstriction. However, relations between these nerves and the proposed ganglion-like elements seem to have been left out of much attention. In the present experiment, it was ascertained that an elevation in the renal perfusion pressure in-

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duced by renal nerve stimulation was effectively blocked by phenoxybenzamine, but not by C₆ and that the rise induced by splanchnic nerve stimulation was prevented either by phenoxybenzamine or C₆. However, in a cross-circulation preparation, in which the recipient's vascular isolation and nervous connection between the kidney and the body were fully maintained, C₆ given into the renal artery failed to block the splanchnic nerve stimuli. Thus it is obvious that the site of blocking action of C₆ is located at the extrarenal area. Probably, the ganglion-like elements had no synaptic function of renal nerves. However, the finding that the renal denervation resulted in a decrease of response to ganglionic stimulants suggests that the renal nerves play a role in synthesizing or storing catecholamines in these elements.

**Summary**

Renal vascular responses to ganglionic stimulants and to renal nerve stimulation were studied in the dog anesthetized with pentobarbital sodium.

1) Ganglionic stimulants, nicotine, DMPP and acetylcholine in the presence of atropine, when given into the renal artery elicited a biphasic elevation in renal perfusion pressure. The first rise in perfusion pressure was completely blocked by phenoxybenzamine and by C₆, but not by adrenalectomy. The second rise and a concomitant elevation in the systemic blood pressure were abolished by these two agents as well as by adrenalectomy.

2) Reserpination of the dog resulted in a marked reduction in the biphasic response thus developed, especially in the first response. Tyramine no longer had a renal vasoconstrictor action, but responses to catecholamines were potentiated.

3) On the seventh to fourteenth day after renal nerve section both tyramine and DMPP lost their immediate vasoconstrictor action.

4) Electrical stimulation of the renal nerve or the splanchnic nerve produced a monophasic or a biphasic elevation in renal perfusion pressure. Such elevations were fully blocked by phenoxybenzamine or C₆ as was the case with ganglionic stimulants. However, the blocking site of C₆ for splanchnic nerve stimulation was found to be in the extrarenal synapse when a cross-circulation preparation was employed.

5) Perfusion of the kidney with the anoxic blood resulted in a marked reduction in responses to ganglionic stimulants, but no or slight reduction in responses to nerve stimuli.

The present data indicate that the biphasic response to ganglionic stimulants is mediated through catecholamines, released from the kidney accounts for the development of the initial response and released from the adrenal gland is attributable to the second response. The catecholamine releasing mechanism of ganglionic stimulants differs from that of tyramine. It is postulated that certain elements, with ganglionic function, even in the absence of anatomical evidence exists in the dog's renal vessels and that the elements have no synaptic function.

**References**


