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THE EFFECT OF EFFERENT DISCHARGES IN RENAL NERVES ON THE ACTIVITY OF ARTERIAL MECHANORECEPTORS IN THE KIDNEY IN RABBIT

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SUMMARY

1. The effect of sympathetic efferent flow in renal nerves on afferent discharges from renal arterial mechanoreceptors was observed in dissected filaments of renal nerves in the rabbit.

2. An increase in afferent discharge rate was observed after renal nerve stimulation.

3. Perfusate accumulated during renal nerve stimulation in an isolated kidney preparation showed a vasoconstrictor effect on blood vessels in rabbit ear.

4. Infusion of a small amount of (±)-noradrenaline increased afferent discharge rate.

5. Modification of blood pressure levels by chemical means modified renal afferent discharge rate.

6. It is suggested that there is an efferent control mechanism on the activity of renal arterial mechanoreceptors by a vasomotor reflex.

INTRODUCTION

In the previous paper (Niijima, 1971), it was reported that there are mechanoreceptors around the renal arterial walls which are sensitive to change in the renal arterial pressure.

It has been reported that vasoconstriction of the renal artery is evoked by stimulating the renal nerves (Aukland, 1968; Block, Wakim & Mann, 1952; Houck, 1951; Study & Shipley, 1950) and by reflex excitation of renal vasoconstrictors (Hix, 1958). Further, a reflex decrease or increase in renal sympathetic discharge was observed by Kezdi & Geller (1968). In 1960, Hunt described the effects of stimulation of the sympathetic trunk on muscle spindles in the cat. He stated that repetitive sympathetic stimula-

tion resulted in an initial lowering of threshold to stretch followed by an increase of threshold. Recently Sampson & Mills (1970) reported that electrical stimulation of cervical sympathetic nerves increased afferent impulse frequency of baroreceptors in the carotid sinus.

From these reports it is reasonable to assume that excitation of renal vasoconstrictors caused by electrical stimulation of renal nerves or reflex facilitation of firing of renal vasoconstrictor cells innervating smooth muscles of renal arteries might have some effect on the activity of mechanoreceptors located around the renal arterial walls. To test this assumption, experiments were conducted on the rabbit's kidney.

METHODS

Experiments were conducted on thirty-five adult rabbits of both sexes. The animals were anaesthetized with urethane (1 g/kg) injected s.c. Two different types of experiment were conducted: experiments on isolated kidney preparations, and experiments *in vivo*. Only left kidneys were used for the experiments because the length of the renal nerve trunks were greater than those of right kidneys. Isolated kidneys were perfused by the method described in a previous paper (Niijima, 1971). In the *in vivo* experiments, arterial blood pressure was recorded from a polyethylene catheter placed in the cardiac end of the common carotid artery. The drugs used were (−)-adrenaline (Sankyo), (±)-noradrenaline (Sankyo) and acetylcholine chloride (Daiichi).

Afferent impulse discharges, single or multi-unit, were recorded from fine filaments dissected from renal nerve trunks. Usually, there are two renal nerve trunks running along the renal artery in the rabbit. One trunk was used for recording and another for stimulation. In experiments conducted *in vivo*, recordings were taken from the peripheral portions of renal nerve filaments after their central connexions were severed. Discharges were recorded through a condenser-coupled differential amplifier (time constant, 0·003 sec) with a kymograph camera and stored on magnetic tape. All analysis of nervous activity took place after conversion of raw data to standard pulses by a sensitive window discriminator which picked up unit discharges from the background noise. The standard pulses were then fed to a small digital computer for further analysis. On-line analyses of discharges were also made.

Square-wave electrical pulses 1 msec duration, 30 Hz but different voltage in separate experiments were applied to the peripheral stump of one of the renal nerve trunks through a pair of silver wire electrodes which were connected through an isolation unit to a stimulator (Nihonkoden, MSE-3).

To detect the substance liberated from the nerve terminals into the renal perfusion fluid during renal nerve stimulation, a bio-assay method was used. A catheter was inserted into the posterior auricular artery of an isolated rabbit ear. The ear was perfused with oxygenated Locke solution having the following composition (NaCl, 154·0 mM; KCl, 2·7 mM; $CaCl_2$, 1·8 mM; $NaHCO_3$, 2·4 mM; glucose, 5·6 mM; pH was 7·4 –7·5), the perfusion fluid entered the posterior auricular artery at about 25° C, pressure 60 cm H_2O and emerged from the superficial temporal vein. The intervals between the drops of perfusion fluid which emerged from the vein were measured by means of a drop counter, which was also used for perfusion experiments on isolated kidneys in which the vasoconstrictor effect of renal nerve stimulation was studied.

RESULTS

The effect of electrical stimulation of renal nerves on afferent discharges from renal arterial mechanoreceptors. In the first experiments, afferent impulse discharges from renal artery mechanoreceptors were recorded *in vivo*. After stimulation of the peripheral part of a cut renal nerve trunk (9 V) for 30 sec, an increase in the afferent discharge rate was observed. It

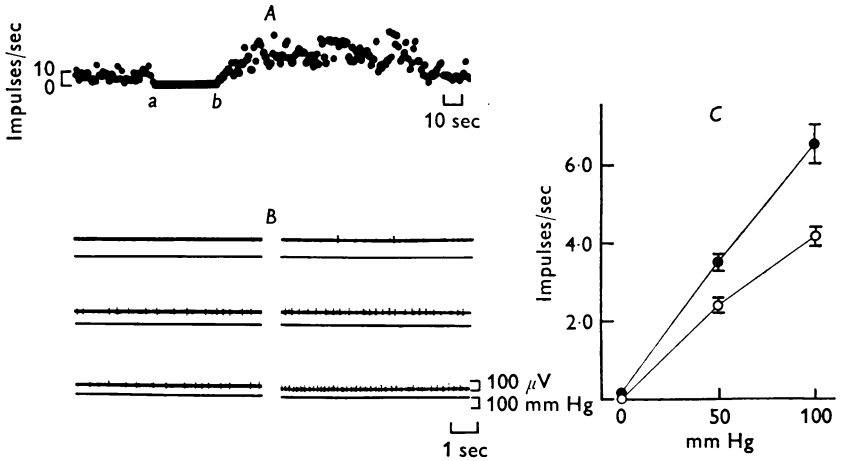

Fig. 1. Effect of renal nerve stimulation on afferent discharge rate of renal artery mechanoreceptors.

A, experiment *in vivo*. Afferent discharges were recorded from one renal nerve and stimuli were delivered to another renal nerve. Stimulation parameters; 9 V, 30 Hz, 1 msec width, 30 sec duration. Afferent discharge rate was plotted against time. Electrical stimuli were delivered from the time marked *a* to *b*. During this period, recording was stopped because of shock artifacts.

B, afferent impulse discharge from renal artery mechanoreceptors at various levels of perfusion pressure before and after renal nerve stimulation. Isolated kidney. Left, before stimulation; right, after stimulation. Stimulation parameters; 2·5 V. 30 Hz, 1 msec and 10 min. Perfusion pressure: from top to bottom, 0, 50 and 100 mm Hg.

C, relationship between afferent discharge rate and perfusion pressure before and after renal nerve stimulation. Isolated kidney. Abscissa, perfusion pressure; ordinate, afferent discharge rate. Each symbol shows the mean of twenty measurements of afferent discharge rate with ± S.E. of mean value. ○ before stimulation ● after stimulation.

lasted about 100 sec and gradually decreased to that of the control level (Fig. 1*A*). When the stimulus strength was weaker, the increase in discharge rate was smaller and of shorter duration. These effects of stimulation were observed in fourteen trials on two rabbits.

Next, experiments were conducted on isolated kidneys to study the

effect of renal nerve stimulation on the relationship between perfusion pressure and afferent discharge rate. Connective tissue surrounding the renal nerve trunks were removed, which made it possible to deliver stimuli with low strength and to dissect single fibres for recording. To obtain a long-lasting vasoconstrictor effect, the perfusion system was clamped on the arterial side for 10 min while electrical stimuli were delivered (2·5 V). Control studies made on isolated kidney preparations from ten rabbits indicated that constant firing rates could be maintained after 10 min of clamping if units were selected which had initially low firing rates and if the temperature of the perfusion solution was kept at 19–20° C. This temperature, lower than in other experiments, was selected in order to prevent the increase in discharge rate due to anoxia during the clamping. Only units which showed no significant change in firing rate during the control of clamping were used in the renal nerve stimulation study.

Before stimulation the relationship between perfusion pressure and afferent discharge rate was observed as a control, and immediately after the electrical stimulation the relationship was observed again. As shown in Fig. 1*B*, there was an increase in discharge rate with an increase in perfusion pressure. This relationship changed after the stimulation as demonstrated in Fig. 1*C*. Before stimulation, the mean discharge rate was 0 impulses/sec at 0 mm Hg, 2·40 impulses/sec at 50 mm Hg and 4·15 impulses/sec at 100 mm Hg. These mean values of afferent discharge rate were obtained from twenty measurements of 20 sec epochs. Just after stimulation, the discharge rate was 0·15 impulses/sec at 0 mm Hg, 3·50 impulses/sec at 50 mm Hg and 6·50 impulses/sec at 100 mm Hg. The plot of discharge rate against perfusion pressure becomes steeper after the stimulation (Fig. 1*C*). Experiments performed on four other kidneys showed similar results.

Vasoconstrictor effect of renal nerve stimulation in the kidney. The vasoconstrictor effect of renal nerve stimulation was studied in three isolated kidney preparations. The kidneys were perfused from the renal artery to the renal vein with oxygenated Locke solution at 70 cm H_2O, and the number of drops emerging from the renal vein was counted every 10 sec. In the experiment of Fig. 2, before renal nerve stimulation the number of drops was 8/10 sec. 5 V pulses were then delivered to the peripheral end of all renal nerve trunks for 5 min. After the onset of stimulation, the number of drops gradually decreased to a minimum value (2/10 sec) 110 sec later, and then increased to the end of stimulation. After cessation of stimulation, it continued to increase and returned to control levels several minutes later. Essentially similar results were observed on two other kidneys during renal nerve stimulation.

Detection of a vasoconstricted substance liberated from nerve terminals

during renal nerve stimulation. In three further experiments the same conditions of perfusion were used, and during renal nerve stimulation (14 V), perfusion fluid emerging from the renal vein was collected in a glass tube. The vasoconstrictor effect of the collected perfusate was tested in the rabbit ear preparation. About 40 sec after the injection of 2 ml. of perfusate, drop intervals started to increase from the control value (0·66 sec). They reached their maximal value (1·91 sec) after 90 sec, and then decreased until they

Fig. 2. Vasoconstrictor effect of renal nerve stimulation. Isolated kidney perfused with Locke solution. Drops emerging fom renal vein were counted for each 10 sec by a drop counter. 10 drops contained 0·46 ml. perfusion solution. All renal nerve trunks were stimulated at peripheral cut end. Stimulation parameters, 5 V, 30 Hz, 1 msec and 5 min. Horizontal line (ST) shows the period of stimulation.

returned to the control value in several minutes. Injection of 2 μg (±)-noradrenaline in 2 ml. Locke solution produced the same effect, but injection of 2 ml of Locke solution, 0·2 μg (±)-noradrenaline in 2 ml. Locke solution or 2 ml perfusate collected before stimulation, produced no effect (Fig. 3). The perfusate collected from two other kidneys during stimulation also caused a similar increase in drop intervals.

The effect of noradrenaline on the afferent discharge rate. One rabbit was used to study the effect of noradrenaline on the afferent discharge rate of renal artery mechanoreceptors. Close arterial injections were made from a catheter in the abdominal aorta which extended to the level of the renal arteries. Two injections of 5 μg and three of 10 μg (±)-noradrenaline (1 ml.) all caused a great increase in discharge rate, while seven injections of 1 μg noradrenaline (1 ml.) and seven of 1 ml. Locke solution were without effect (Fig. 4*A*).

In further experiments five isolated kidneys were perfused by a system which permitted a rapid switch between Locke solution and test solutions, which contained 5 μg or 10μg (±)-noradrenaline/l. In the preparation shown

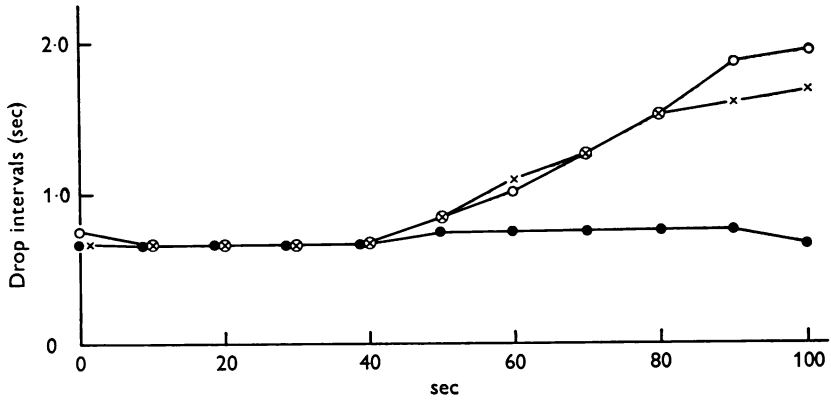

Fig. 3. Vasoconstrictor effect of perfusate from the kidney collected during renal nerve stimulation. Isolated kidney. Drop intervals of perfusion solution emerging from veins in rabbit ear plotted against time. 2 ml. test solution was injected into the rubber tube which connects the reservoir and auricular artery at 10 in time base. ○, perfusate accumulated during renal nerve stimulation. ×, 2 μg (±)-noradrenaline in 2 ml. Locke solution. ●, 0·2 μg (±)-noradrenaline in 2 ml. Locke solution.

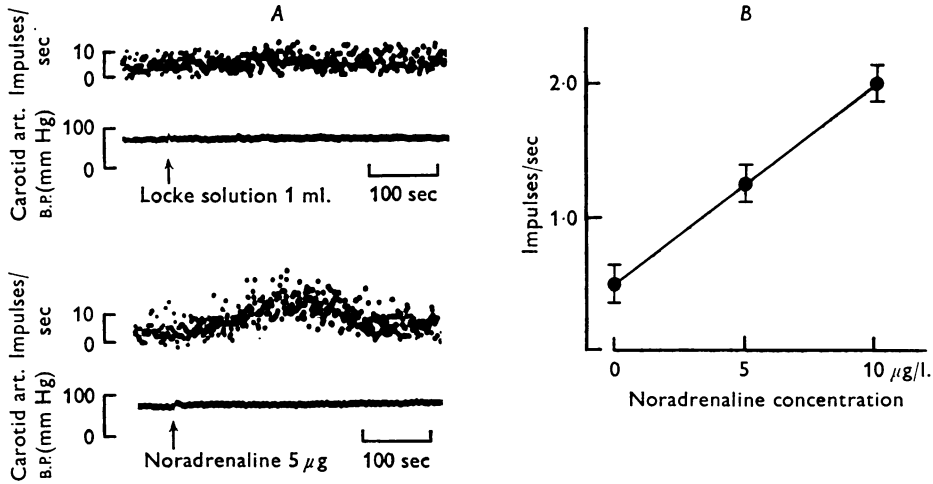

Fig. 4. Effect of noradrenaline on the afferent discharge rate.

A, experiment *in vivo*. Close arterial injections were made from a catheter in the abdominal aorta. Upper traces in each Figure show afferent discharge rate. Lower curves in each Figure show systemic blood pressure. Arrow shows the time of injection.

B, relationship between noradrenaline concentration (abscissa) and afferent discharge rate (ordinate). Isolated kidney. Each point shows mean value with ± S.E. of mean (N = 20).

in Fig. 4*B*, the mean discharge frequency in the control experiment was 0·50 impulses/sec. It increased to 1·25 impulses/sec after switching to 5 μg (±)-noradrenaline/l. and to 1·58 impulses/sec after switching to 10 μg/l. Similar results were observed on the other four kidneys.

The effect of vasomotor reflexes on afferent discharge rate in vivo. Six rabbits were studied. The left kidney was perfused by oxygenated Locke solution through a catheter inserted into the renal artery, and the solution emerged through the cut renal vein. Recordings were made from a filament dissected from the peripheral cut end of one renal nerve trunk and the other

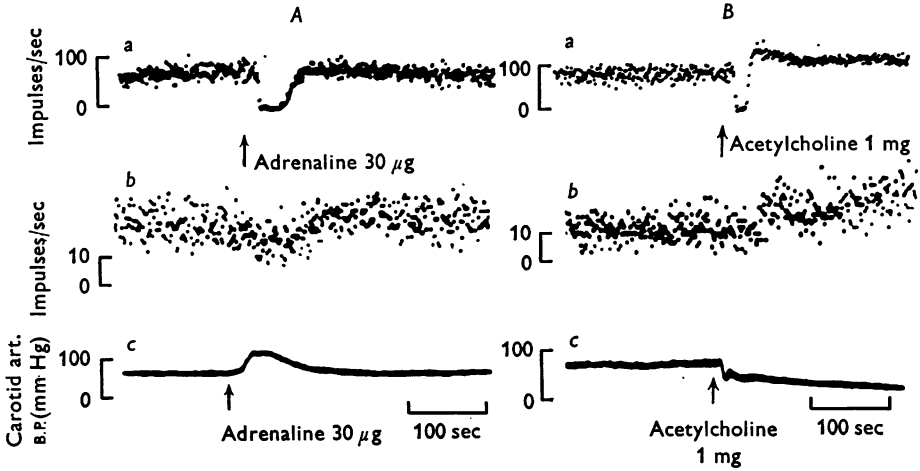

Fig. 5. Effect of vasomotor reflex on afferent discharge rate. Experiments *in vivo*. *a* in each Figure shows efferent discharge rate recorded from central stump of a renal nerve bundle. *b* in each Figure shows afferent discharge rate recorded from peripheral stump of renal nerve filament. *c* in each Figure shows systemic blood pressure. Traces in *b* and *c* were recorded simultaneously in the same animal. Traces in *a* were recorded from another animal. In *A*, arrows indicate infusion of 30 μg (−)-adrenaline. In *B*, arrows indicate infusion of 1 mg acetylcholine. Blood pressure curves recorded with traces in *a* were omitted because they have the same time course as those recorded in trace *c*.

renal nerve trunk was kept intact. In one rabbit (Fig. 5*A*) a depression of afferent discharge rate was observed after I.V. injection of 30 μg (−)-adrenaline, which caused an increase in systemic blood pressure. Intravenous infusion of the same dose of (−)-adrenaline into another rabbit depressed efferent discharges recorded from the central cut end of a renal nerve bundle (Fig. 5*A*). Acetylcholine 1 mg I.V. in the same two animals caused an initial depression followed by a long lasting increase in efferent discharge rate with coincident blood pressure depression, and (Fig. 5*B*) an increase in afferent discharge rate. In four further animals a similar

relationship between blood pressure and afferent discharge rate was observed.

DISCUSSION

The activity of the arterial mechanoreceptors increased after stimulation of the peripheral stump of the renal nerve. This long-lasting excitatory effect of renal nerve stimulation might be interpreted as a result of a chemical transmitter liberated from the nerve terminals during stimulation. In the perfusion experiments, it was confirmed that renal nerve stimulation caused vasoconstriction, inferred from diminished blood flow emerging from the renal vein. Perfusate collected from the renal vein during renal nerve stimulation showed a vasoconstrictor effect on the blood vessels of the rabbit ear. The most likely candidate for the substance causing this vasoconstriction is noradrenaline which was released from sympathetic nerve terminals in the kidney. There are several reports of vasoconstrictor effect of sympathetic nerve fibres in the renal nerve excited by reflex or direct electrical stimulation of the renal nerve (Aukland, 1968; Block *et al.* 1952; Hix, 1958). As another possible substance, renin secreted by nerve excitation can be considered (Vander, 1965).

Perfusion of the isolated kidney with noradrenaline in Lock solution also caused an increase in receptor activity. A similar result was reported by Landgren, Neil & Zotterman (1952) on carotid baroceptors. A possible interpretation is that the receptor activity was increased by an increase in arterial wall tension which followed a rise in perfusion pressure, or by muscular contraction of arterial walls, but the possibility of direct sensitization of mechanoreceptors by noradrenaline cannot be eliminated. Iggo (1955) reported that gastric mechanoreceptors and mechanoreceptors in the wall of the urinary bladder were tension receptors 'in series' with muscle fibres. As there is an increase in firing rate during passive distension and active contraction of the renal arterial wall, afferent discharge from renal arterial mechanoreceptors could be interpreted in this light.

The experiments shown in Fig. 5 indicate that an increase in blood pressure after an injection of adrenaline caused a reduction in afferent discharge rate, a decrease in blood pressure after an injection of actylcholine an increase. Further, an increase in blood pressure after adrenaline infusion caused a depression of efferent discharge in the renal nerve and a decrease in blood pressure after acetylcholine infusion caused an increase. These responses might be due to a vasomotor reflex from baroreceptors in the carotid sinus and aortic arch (Ninomiya & Irisawa, 1969), but the effect of increase or decrease in oxygen supply to spinal cardiovascular centres due to the rise or fall in blood pressure should also be considered (Alexander, 1945). The experiments shown in Fig. 5 support the idea that the activity

of mechanoreceptors around renal arterial walls is controlled by renal sympathetic efferents because the kidney was perfused by Locke solution through the renal artery, and the renal nerve bundles were the only connexions with the body. Even in this condition, adrenaline injected into the jugular vein resulted in a decrease and acetylcholine caused an increase in afferent discharge rate.

It is suggested that there is a mechanism which controls the activity of renal arterial mechanoreceptors through a renal vasomotor reflex.

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