The recent development in biochemical physiology has established the concept of circulatory mediator substance which probably regulates the circulation in each peripheral organ in different modes. The postocclusive vascular response is used for the purpose of elucidating this regulatory mechanism in expectation of accumulation of such substance either in the tissue or in the venous blood. Concerning the renal tissue, Bier (1898) reported the absence of vasodilatory response after the release of occlusion of the renal artery. This characteristic feature of response has absorbed the attention of many physiologists because in other organs a prominent vasodilation is commonly observed, generally known as the reactive hyperemia. Further studies, however, showed complexity of the renal vascular response after the release of occlusion and reported that not only absence of response, but also vasoconstriction were induced as the postocclusive vascular response of the renal artery. Furthermore even vasodilation was not rarely observed although the type of vasodilation was different from that of the coronary and femoral artery.

In this paper our study is focused on the elucidation of the mechanism of the peculiar vasoconstrictory response called the reactive ischemia. Angiotensin may be the due substance of the postocclusive vasoconstriction of the renal circulation, because the quick response in the release of renin from the tissue into the renal artery is observed either by the local ischemia or by the selective administration of noradrenaline, and renin converts inactive angiotensinogen to active angiotensin in the renal vasculature. However, there are other probabilities such as the release of catecholamine or adenosine. In fact, the renal artery belongs to the most reactive artery to catecholamine, and adenosine is found to be the specific renal vasoconstrictor. It is
suggested that the reactive ischemia of the renal circulation may be induced by an interaction of catecholamine and adenosine but not by the renin-angiotensin system.

METHODS

Forty five adult mongrel dogs, both sexes, weighing around 10 kg were used, nine of which were reserpinized with daily subcutaneous dose of 0.25 mg/kg for 2 days before the experiment. Nontreated animals were anesthetized with i.v. sodium pentobarbital (30 mg/kg), but for reserpinized animals a single dose of 20 mg/kg was usually enough for anesthesia. The animal was placed left side up on the operating table. The left kidney was exposed retroperitoneally through the wide incision along the costal arch, and the renal artery was carefully isolated from the surrounding tissue to avoid injury to the nerve fibers as far as possible. The arrangement of the renal perfusion system was described in the diagram of Fig. 1. Sodium heparin (whale origin) was given initially in a dose of 500 U/kg. The femoral arteries of both sides were cannulated. The blood led from each artery was brought together in one channel and then it was driven by the Sigmamotor pump (Model T8) into the left renal artery. The perfusion pressure was adjusted to about 100 mm Hg at the beginning of experiment. Hereafter the constant renal blood flow was maintained by the constant pumping volume throughout the experiment. Three way stopcock (TC) was arranged close to the renal arterial cannula between the cannula and the Sigmamotor pump, one way of which was connected to either the left or the right femoral vein to shunt the blood during the arterial occlusion, so that the fresh arterial blood could be supplied as soon as the occlusion was released. The duration of arterial occlusion was arranged for 1 or 2 minutes. The mean systemic blood pressure (SBP) and the perfusion pressure

![Diagram of the renal perfusion system](image)

**Fig. 1.** Diagram of the renal perfusion system. SBP; transducer for measuring the systemic blood pressure, pump; Sigmamotor pump, TC; three way stopcock, PP; transducer for measuring the perfusion pressure, F; electromagnetic flowmeter, A; from the femoral artery and V; to the femoral vein.
RENAL REACTIVE ISCHEMIA

(PP.) were measured by the pressure transducer (Nihon Kohden MP-4T). The renal blood flow was measured by an electromagnetic flowmeter (Nihon Kohden MF-2T). An ink writing oscillograph was used for recording. The ordinary experimental course was finished within 3 hours.

Since the renal blood flow was constant throughout the experiment, the change in the perfusion pressure was proportional to the change in the resistance. Thus the vascular resistance after the release of occlusion was readily calculated and its maximum change was expressed as a percentage of the preocclusive value (Max % Δ in R.).

Drugs used in the experiment were dl-noradrenaline hydrochloride (Sankyo), acetylcholine hydrochloride, (Daiichi), tyramine hydrochloride and serotonin creatinine sulfate (Daiichi Kagaku), atropine sulfate (Takeda), hexamethonium bromide and tolazoline hydrochloride (Yamanouchi), angiotensin II (Ciba), phenoxybenzamine hydrochloride (SK & F), dipyridamole (Boehringer Ingelheim), reserpine (Ciba), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenosine (kindly supplied by Waldhof in Germany), inosine monophosphate (IMP), hypoxanthine and inosine (kindly supplied by Dr. Y. Aramaki, Takeda Pharmaceutical Co. Laboratories).

Each drug was dissolved in saline as the stock solution which was diluted freshly with saline just before use. The drug solution was injected into a rubber tube connected close to the arterial cannula. The injected volume was 0.1 ml in a period of 10 seconds. The continuous administration of drug was performed by Harvard infusion pump (Harvard apparatus Model 600-900).

RESULTS

1) Characteristics of the postocclusive vascular response (Table 1. Figs. 2 and 3)

The postocclusive vascular response of the renal artery was modified by the duration of occlusion time. As shown in Fig. 2, the reactive ischemia

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Ratios of a) reactive ischemia, b) reactive hyperemia and c) dumb response as the pattern of the renal postocclusive vascular response of the renal artery in nontreated dogs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of experiments</td>
<td>Occlusion time (min)</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>2</td>
</tr>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
was clearly observed after 1 or 2 minutes. However, it steadily diminished after the longer duration of occlusion time and the long lasting reactive hyperemia took place. However, the pattern of 1 or 2 minutes arterial occlusion was almost the same for each animal during the course of experiment. 

Fig. 2. Typical records of the postocclusive renal vascular responses related to the length of occlusion time.

Fig. 3. Patterns of postocclusive vascular response. A; reactive ishma, B; reactive hyperemia and C; dumb response.
majority of the renal vascular response after the release of occlusion was the vasoconstriction which was called the "reactive ischemia" (TABLE 1). The definite increase of resistance was observed immediately after the reopening of the perfusion system and it returned to the initial level with or without a marked hyperemia (FIG. 3, A). The minor type of response was the vasodilation which might belong to the general term of the "reactive hyperemia", but the grade of dilation was significantly less than that in the coronary or the femoral artery (FIG. 3, B). Finally there remained a rather rare type of response which reacted with neither ischemia nor hyperemia, that is to say, dumb response as shown in FIG. 3, c.

2) Postocclusive vascular response in the reserpinized animals (TABLE 2).

In the cases of the reserpinized dogs, the reactive ischemia occurred at a lower frequency (TABLE 2) than that of nontreated dogs (TABLE 1). This difference was not so definite, which might be due to the insufficient amount of reserpine used for depleting catecholamine from the renal tissue, because 30 µg of tyramine still caused a rise in the perfusion pressure.

3) Effects of various drugs on the reactive ischemia.
a) Effects of atropine, hexamethonium and tolazoline (FIG. 4). Atropine (1 mg), hexamethonium (2.5 mg) and tolazoline (1 mg) were given intraarterially. These drugs induced a temporal response of the vasoconstriction. Thus, the arterial occlusion was performed just when the perfusion pressure returned to the initial level. Atropine and hexamethonium did not modify the course

<table>
<thead>
<tr>
<th>Number of experiments</th>
<th>Occlusion time (min)</th>
<th>a Increased resistance</th>
<th>b Decreased resistance</th>
<th>c No change</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>1</td>
<td>33 (67)</td>
<td>44 (29-125)</td>
<td>23 (4-24)</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>44 (56)</td>
<td>45 (29-49)</td>
<td>11 (2-20)</td>
</tr>
</tbody>
</table>

TABLE 2.
Ratios of a) reactive ischemia, b) reactive hyperemia and c) dumb response as the pattern of the postocclusive vascular response of the renal artery in reserpinized dogs. Daily dose of 0.25 mg/kg of reserpine was given subcutaneously for 2 days before the experiment.
of the vascular response while they blocked the action of ACh (10 µg) and nicotine (5 µg) respectively. Tolazoline was found to have a partial inhibitory effect on the postocclusive vascular response while it blocked completely the action of noradrenaline (0.1 µg).

b) Effect of phenoxybenzamine (Table 3, Figs. 5 and 6). Five mg of phenoxybenzamine was dissolved in 10 ml of saline and infused continuously into the renal artery for 20 minutes at the rate of 0.5 ml/min. The vasodilation was observed clearly by phenoxybenzamine treatment, and the perfusion pressure decreased from 100 mmHg to 70 mmHg more or less. The arterial occlusion was repeatedly performed for 1 hour after the completion of infusion. The postocclusive vasoconstriction became less prominent (Table 3) and finally it disappeared as shown in a typical result of Fig. 5. The response of noradrenaline (0.1 µg) was completely blocked but the actions of 5-HT and adenosine were rather potentiated (Fig. 6), which might be due to the loss of vascular tone by phenoxybenzamine treatment.

c) Potentiation of the vasoconstriction by dipyridamole (Table 4, Fig. 7).

![Figure 4. Effects of atropine, hexamethonium and tolazoline on the postocclusive vasoconstriction.](image)

<table>
<thead>
<tr>
<th>Table 3. Maximum per cent change in the renal vascular resistance (Max. % ( \triangle ) in R.) after the treatment of phenoxybenzamine.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Number of experiments</td>
</tr>
<tr>
<td>Max. % ( \triangle ) in R Mean (Range)</td>
</tr>
</tbody>
</table>
Dipyridamole is a potent coronary vasodilator but it selectively constricts the renal artery. This drug potentiated strikingly the postocclusive vasoconstriction, and the response obtained was about 3 times as large as the control response before drug administration. The result is summarized in Table 4. The drug potentiated, not only the action of adenosine and AMP but also

![Graph](image1)

**Fig. 5.** Prevention of the reactive ischemia by the treatment of phenoxybenzamine. 10 ml of phenoxybenzamine (0.05%) solution was infused intraarterially into the left renal artery in a rate of 0.5 ml per min. for 20 minutes.

![Graph](image2)

**Fig. 6.** Comparison of the postocclusive vascular responses before and 1 hour after phenoxybenzamine infusion. 10 ml of phenoxybenzamine solution was infused intraarterially into the left renal artery in a rate of 0.5 ml per min. for 20 minutes. Effects of noradrenaline (NA), 5-hydroxytryptamine (5-HT) and adenosine on the renal vascular tone were also compared.
TABLE 4.

Effect of dipyridamole treatment on the postocclusive vascular response of the renal artery. Number of experiments was 9. Used doses of dipyridamole were 50 and 100 μg, and occlusion times were 1 and 2 minutes. (Max. % Δ in R. was maximum per cent change in the vascular resistance.)

<table>
<thead>
<tr>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max. % Δ in R. Mean±SE</td>
<td>39.9±6.7</td>
</tr>
</tbody>
</table>

Fig. 7. Potentiation of the reactive ischemia and effects of adenosine, AMP and noradrenaline (NA) by dipyridamole treatment.

that of noradrenaline as shown in Fig. 7. Inosine, IMP and hypoxanthine were ineffective on the renal artery even after dipyridamole treatment.

4) Appearance of reactive ischemia after administration of noradrenaline and/or adenosine (Figs. 8 and 9).

The effects of noradrenaline, adenosine, AMP and angiotensin were tested on the postocclusive vascular responses in 22 cases of dumb response and reactive hyperemia out of 45 animals. Each of these substances was infused continuously into the renal artery for 10 minutes.

The infusion of noradrenaline, the total dose of which was within a range of 10 to 50 μg, induced the prompt rise of the perfusion pressure which was sustained at a certain level during the period of infusion but returned to the initial level when the infusion was stopped. At this stage the arterial occlusion was tested. The postocclusive vasoconstriction, i.e., reactive ischemia, appeared in the cases of dumb response or reactive hyperemia in 8 cases out of 13. The typical result was shown in Fig. 8. The per cent increase of resistance in 8 cases was 22.5±4.62 (mean and standard error). In 4 cases out of 5, which did not convert to the postocclusive ischemia after the infusion of noradrenaline, the successive infusion of adenosine caused the postocclusive
ischemia. The increase of resistance was 22.5 (16 to 33) per cent of the initial value (mean and range). One case did not change its vascular response even after the successive infusions of noradrenaline and adenosine.

The infusion of adenosine produced a distinct vasoconstriction at the beginning of infusion but the constriction was not sustained. The postocclusive vasoconstriction appeared in 4 cases out of 9. The increase of resistance was 21 (16 to 30) per cent (mean and range). In 4 cases out of 5 which did not convert to the postocclusive ischemia after the infusion of adenosine, the successive infusion of noradrenaline caused the ischemia after arterial occlusion.

**Fig. 8.** Typical patterns of induction of the reactive ischemia in cases of dumb response by infusion of noradrenaline or adenosine but absence of the effect by AMP or angiotensin. Total dose of each compound was described on each curve. The rate infusion was 1 ml per min. for 10 minutes on each solution.

**Fig. 9.** Induction of the reactive ischemia by alternate infusions of noradrenaline and adenosine. The rate of infusion was 1 ml per min. and total dose was marked on each curve.
The per cent increase of resistance was 25 (18 to 35) in mean and range. One case still remained in the dumb response even after the successive infusions of adenosine and noradrenaline.

AMP (100 to 1000 μg) acted on the renal vascular system as adenosine did, but AMP didn't modify the postocclusive response at all in 4 cases. Angiotensin (1 to 5 μg), caused a potent vasoconstrictory response which was much the same as that induced by noradrenaline infusion. However, the postocclusive vasoconstriction was never induced after angiotensin treatment of 3 cases as shown in Fig. 8.

Once the reactive ischemia occurred after noradrenaline or adenosine infusion in cases of the dumb response or the reactive hyperemia, it could be induced repetitively, though the grade of reactive ischemia was going to diminish. Phenoxylbenzamine treatment was necessary to get initial responses of dumb response or hyperemia again.

DISCUSSION

Not only vasoconstriction but also vasodilation were observed as the postocclusive vascular response of the renal artery in most cases of this study, but a few cases showed no special reaction (dumb response). The ratio of these responses shifted to a higher frequency in the incidence of vasodilation in the reserpinized animals. Moreover, after the treatment of tolazoline the postocclusive vasoconstriction became definitely less prominent than that before the treatment and it was prevented completely by the use of phenoxylbenzamine. The reactive ischemia was induced after the infusion of noradrenaline into the renal artery in either case of dumb response or of reactive hyperemia. These observations strongly suggest that the postocclusive vasoconstriction is mainly developed by the renal sympathetic mechanism.

In connection with the sympathetic mechanism, adenosine must be considered. The infusion of adenosine into the renal artery converted the reactive hyperemia or dumb response to the reactive ischemia. Furthermore the reactive ischemia was potentiated considerably after the selective administration of dipyridamole, a potent compound to prevent the deamination of adenosine. These observations are direct pharmacological evidences to support the participation of nucleoside metabolism in the mechanism of the reactive ischemia.

Both noradrenaline and adenosine are shown to be amenable substances to analyze the mechanism of the reactive ischemia. In this experiment there are some evidences in favour of the authors' working hypothesis of interaction between both substances. After the selective use of dipyridamole, not only adenosine but also noradrenaline were definitely potentiated by the selective injection into the renal artery. Thus the prominent potentiation of reactive
ischemia by the treatment with dipyridamole is probably due to its effect on both nucleoside and catecholamine. Furthermore, the successive infusions of adenosine and noradrenaline could convert the reactive hyperemia or dumb response to the reactive ischemia in a few cases to which single infusion of either adenosine or noradrenaline was not effective for this conversion. In the literature, there are some reports of the interaction between catecholamine and adenine nucleotides. The presence of relatively large amounts of ATP in catecholamine storage granules is found in the adrenal medulla and in adrenergic nerves. The participation of ATP and ADP is observed in the mechanism of catecholamine release and uptake in isolated adrenergic nerve granules. Catecholamine is released from the cardiac tissue by anoxia, while there is no direct information about the catecholamine release as the renal response to the ischemia. If the release of catecholamine accompanied by the discharge of ATP is accepted as the sequence of renal ischemia, above observations in this study are quite understandable, because ATP discharged will be rapidly hydrolysed in the renal vasculature.

In the earlier period of research Conway and Cooke reported a relatively high distribution of adenosine deaminase in the renal tissue. However, Gerlach, Deuticke and Drisbach demonstrated the metabolic pathway of adenine nucleotides in the renal tissue through deamination of AMP to IMP but not through deamination of adenosine to inosine. Furthermore, Gerlach et al. estimated the amount of nucleotides in the renal tissue after ischemia and found no accumulation of adenosine in the renal tissue. Therefore the sole release of adenosine from the ischemic renal tissue may not be expected while the activity of adenosine deaminase is relatively high in the kidney. In this study no effect of AMP infusion was observed for inducing the reactive ischemia in cases of dumb response, while its renal vasoconstrictory effect and the potentiation by dipyridamole were similar to those of adenosine. Ready transfer of adenosine into the vascular smooth muscle may be the cause of the difference in the effect on the reactive ischemia between adenosine and AMP. Adenosine transferred may be utilized for synthesis of ADP and ATP which conjugate with catecholamine in the store site.

Angiotensin infusion caused a very distinct vasoconstriction but did not induce the conversion of the postocclusive vasodilation to the reactive ischemia. This simply suggests that the release of renin from the ischemic renal tissue and sequential production of angiotensin is probably not a principal mechanism of the postocclusive vasoconstriction. No potentiation of angiotensin by dipyridamole treatment is in favour of this conclusion.

SUMMARY

The mechanism responsible for the postocclusive vasoconstriction of the renal artery of dog was analyzed by pharmacological evaluation.
The renal circulation responded variably animal by animal after the release of occlusion. Among 36 nontreated dogs, the vasoconstriction, i.e., "reactive ischemia", the vasodilation, i.e., "reactive hyperemia", and no change in the vascular response, i.e., "dumb response" were obtained after 1 minute occlusion in 53, 33 and 14 per cent respectively. The number of the reactive ischemia among 9 reserpinized animals diminished to 33 per cent, while the number of the reactive hyperemia and dumb response increased to 44 and 23 per cent respectively.

The reactive ischemia was blocked completely by phenoxybenzamine (α-adrenergic blocking agent) treatment, while this phenomenon was potentiated remarkably by the treatment of dipyridamole (adenosine deaminase inhibitor). Dipyridamole treatment potentiated also vasoconstrictory response of adenosine, AMP and noradrenaline.

In cases of absence of the postocclusive vasoconstriction, noradrenaline or adenosine infusion or successive infusions of both substances converted the vascular response to the reactive ischemia, while AMP or angiotensin infusion did not modify the postocclusive vascular response at all.

These results imply that catecholamine-adenosine system would play a dominant role in the mechanism of the postocclusive vasoconstriction of the renal artery.

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