Interaction between Adenosine Compounds and Norepinephrine in Dog Renal Circulation

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HASRIMOTO, K. and KOKUBUN, H. Interaction between Adenosine Compounds and Norepinephrine in Dog Renal Circulation. Tohoku J. exp. Med., 1972, 107 (4), 373-380 — Drugs were administered to anesthetized dogs directly into the left renal artery perfused with animal's own blood at a constant pressure. A single injection of adenosine caused prompt renal vasoconstriction, while an infusion of adenosine caused initial vasoconstriction which recovered to the control level even though infusion was continued. The vasoconstriction caused by a single injection of adenosine was not only depressed but also reverted to vasodilation during infusion of adenosine in its concentration from $8 \times 10^{-9}$ to $8 \times 10^{-6}$ g/ml in the perfused blood (self-inhibition), while norepinephrine-induced vasoconstriction was significantly enhanced in the concentration as low as $3 \times 10^{-9}$ g/ml of adenosine. The vasoconstriction caused by stimulation of renal periarterial nerve fibers was also significantly potentiated. These changes in responses disappeared promptly after interruption of adenosine infusion. Infusion of AMP acted similarly to adenosine but the effects were less. Infusion of ADP and ATP also depressed the effect of adenosine while the potentiation of the effect of norepinephrine was less during ADP infusion and absent during ATP infusion. Adenosine or AMP probably plays an important role in regulation of blood flow by modulating the adrenergic mechanism in the renal circulation.

Since Gordon (1960) observed the release of adenosine compound in the blood stream of the ischemic kidney, much interest has been focused on its probable physiological role in the renal circulation. Adenosine and AMP markedly increase renal vascular resistance (Hashimoto et al. 1964, Hashimoto and Kumakura 1965, Scott et al. 1965), while they are potent vasodilators in other organs. This unique response of renal vasculature to these compounds has stimulated further studies on their possible role in local regulation of the renal blood flow (Harvey 1964, Thurau 1964, Nechay 1966, Haddy and Scott 1968, Tagawa and Vander 1970). Recently, Sakai et al. (1968) observed that postocclusive vasoconstriction in the renal artery was potentiated by treatment with dipyridamole, an agent which enhances the effects of adenosine compounds, while it was blocked by phenoxybenzamine. Furthermore, Hashimoto et al. (1970) reported that renal vasoconstrictor responses to adenosine and also to norepinephrine were potentiated during intra-arterial infusion of dipyridamole.

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In this study, we observed some interesting actions of adenosine compounds which probably modulate the adrenergic mechanism in the renal circulation.

METHODS

Fifty-five adult mongrel dogs of both sexes, weighing 13 to 22 kg, were used. They were anesthetized with sodium pentobarbital, 30 mg/kg i.v. initially and with supplemental doses of 50 mg given by the same route as required. A tracheal cannula was inserted to perform artificial respiration. The carotid artery was cannulated for the measurement of the mean systemic blood pressure. Via flank incision, the left renal artery was exposed retroperitoneally. Care was taken not to injure the visible nerve fibers around the renal artery. Then the artery was ligated and cut from the abdominal aorta together with accompanying nerve fibers, and a polyethylene cannula was quickly inserted into the cut end toward the kidney. Sodium \(\omega\)-heparin (Taiyo Fishery) (Hashimoto et al. 1963) was given initially in a dose of 500 U/kg i.v., with supplemental doses of 1,000 U/hr throughout the experiments as an anticoagulant. In order to maintain the mean systemic blood pressure over 100 mm Hg, heparinized blood was transfused via the jugular vein from time to time. The animal’s own blood, led from the right femoral artery, was made to flow into the left renal artery through a cannulating probe of an electromagnetic flowmeter (Nihon Kohden MF-2) at a constant perfusion pressure of 100 mm Hg. The constant pressure perfusion was attained by use of a pneumatic resistance set in parallel to this circuit and by shunting the excess blood to the femoral vein. The mean systemic blood pressure was maintained at over 100 mm Hg, so the renal perfusion pressure at 100 mm Hg was gained throughout the experiments. Systemic blood pressure and renal perfusion pressure were measured by pressure transducers (Nihon Kohden RP-2), and were recorded continuously on an ink-writing oscillograph (Nihon Kohden WI-180 U). Periarterial nerve fibers were stimulated (10 cps, 1 msec, 4–6 V, for ten seconds) by an electronic stimulator (Nihon Kohden MSE-3R) through silver electrodes attached around the renal artery at a site distal to the arterial cannula.

Drugs used were adenosine (Boehringer & Sohn), adenosine-5’-monophosphate (AMP,

![Diagram of the renal perfusion system](Image)
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Daiichi), adenosine-5'-diphosphate trisodium (ADP, Waldhof) (base), adenosine-5'-triphosphate disodium (ATP, Sigma) (base), and dl-norepinephrine hydrochloride (Sandoz) (base). Drugs were dissolved in 0.9% saline as stock solutions and were diluted with 0.9% saline just before use. The drug solutions were injected into the rubber tube close to its connection with the renal arterial cannula. The volume was fixed at 0.1 ml and injected in a period of ten seconds. Control injections of 0.9% saline had no effect on blood flow. For continuous administration, an infusion pump (Harvard Apparatus Model 600-900) was used, adjusted to deliver drug solutions at the rate of 0.1 ml per minute.

For the quantitative comparison of vasoconstrictor responses the figure obtained by multiplying the change in blood flow by half its duration was used. The effects of adenosine, norepinephrine and nerve stimulation during infusion of adenosine compounds are expressed as percentage decreases or increases of the control values.

Results

The rate of renal blood flow

The average blood flow in the left renal artery was 122.4±9.1 (mean and S.E.) ml/min (n=55). The rate of infusion of drugs was from 0.3 ng to 1 mg/min, so the concentration of a drug in the perfused blood varied from approximately 3×10⁻⁹ to 8×10⁻⁶ g/ml.

Effects of infusion of adenosine

Fig. 2 illustrates typical features of responses of the renal artery to adenosine, norepinephrine and periarterial nerve stimulation before, during and immediately after infusion of adenosine. A single injection of adenosine into the renal artery resulted in marked vasoconstriction as did norepinephrine or perivascular nerve stimulation. When adenosine was infused intra-arterially, the blood flow decreased immediately and then recovered gradually toward the initial level while the infusion was continued. When the infusion rate was higher, the initial decrease in blood flow was more pronounced and the time required for recovery was shorter. At above 100 ng/min, the blood flow not only recovered to the level before infusion within about five minutes but increased above the control level, that is, renal vasodilation was induced. During infusion of adenosine, the vasoconstrictor effect of adenosine from a single injection was inhibited. The data are summarized in Table 1 (A). Such self-inhibition of adenosine was clearly observed at the infusion rate of 10 ng/min which corresponded approximately to 8×10⁻⁸ g/ml, and it was almost complete at the rate of 0.1 to 1 mg/min (8×10⁻⁷ to 10⁻⁶ g/ml). As shown in the bottom curve of Fig. 2, the response to a lower dose (10 ng) of adenosine was completely blocked, while that to a larger dose (100 ng, 1 mg) of adenosine was converted to vasodilation at the infusion rate of 100 ng/min (approximately 8×10⁻⁷ g/ml).

The vasoconstriction induced by norepinephrine was markedly enhanced by infusion of adenosine (Fig. 2, Table 1 (A)). The dose of norepinephrine given was 0.5 or 1 µg. Even with 0.3 µg/min of adenosine (approximately 3×10⁻⁹ g/ml), the percentage increase of the response to norepinephrine was 44.9±4.0 (p<0.01), and it became 207±12 with 100 µg/min of adenosine. Vasoconstriction induced by
periarterial nerve stimulation was also potentiated (53.8±10.3%) during infusion at this rate. The increase over the control response was statistically significant (p<0.01).

Responses to adenosine, norepinephrine and nerve stimulation all recovered promptly to their initial values after cessation of adenosine infusion (Fig. 2).

**Effects of infusion of AMP**

The potency of the vasoconstrictor effect of a single injection of AMP was about 80% of that of adenosine. Infusion of AMP also induced an initial decrease in blood flow, and during the steady state the responses to single injections of adenosine and norepinephrine altered in the same way as during adenosine infusion but AMP was a little less potent. The data are summarized in Table 1 (B). Percentage decrease in responses to a single injection of 10 µg of adenosine was statistically significant at an infusion rate of from 10 µg to 1 mg/min of AMP. These changes are smaller than those caused by infusion of adenosine but the difference is not statistically significant (0.05<p). During infusion of AMP, the response to 0.5 or 1 µg of norepinephrine was also potentiated significantly with infusion rate of 1 µg to 1 mg/min, but the change was smaller than that occurring during adenosine infusion.
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**Effects of infusion of ADP**

ADP administered by a single injection into the renal artery produced different effects with different doses, i.e., vasoconstriction with a smaller dose (1 to about 30 μg) and vasodilation with a larger dose (100 μg to 1 mg), but when infused into the renal artery it always caused an increase in resistance. The initial decrease in blood flow, however, was smaller than that induced by infusion of adenosine or AMP. It quickly recovered to the control level within 1 to 5 min. Percentage changes in responses to adenosine and norepinephrine during ADP infusion are summarized in Table 1 (C). ADP inhibited the vasoconstriction induced by adenosine and enhanced that by norepinephrine, and the changes were statistically significant at above 100 μg/min. With regard to both effects, ADP was less potent than adenosine or AMP.

**Effects of infusion of ATP**

ATP was the only adenine nucleotide that caused constantly vasodilation in the renal vasculature. Infusion of ATP at the rate of 1 mg/min increased renal blood flow about 17%. Responses to adenosine were significantly reduced at infusion rates greater than 100 μg/min. Changes in responses to nore-
pinephrine were not statistically significant with any infusion rates tested (Table 1 (D)).

**Discussion**

The selective administration of adenosine or adenine nucleotides into the renal artery by infusion in a concentration of approximately $8 \times 10^{-9}$ to $10^{-6}$ g/ml inhibited the vasoconstrictor effect of adenosine while it potentiated that of norepinephrine. Among these adenine compounds, adenosine was the most potent in both actions. In this study we term the former effect 'self-inhibition' of adenosine. Previously Gaddum (1953) observed such desensitization to 5-HT in the contractions of guinea pig ileum which had previously been treated with 5-HT, and he suggested that 5-HT occupied the site of action thus blocking the action from the additional administration of 5-HT. On this assumption, adenosine infusion might occupy the site of its vasoconstrictor action with the result that 'self-inhibition' occurred. Furthermore, we observed the vasodilating effect of adenosine, given in larger doses, after an initial decrease in flow reverted to the control level by 'self-inhibition' during continuous infusion. Thus, a dual effect of adenosine, vasoconstriction and vasodilation in the renal circulation, was clearly demonstrated in these experiments. The latter effect is unveiled when the site responsible for vasoconstriction is occupied.

The vasoconstriction induced by norepinephrine was markedly enhanced by infusion of adenosine even in the low concentration of $3 \times 10^{-9}$ g/ml in the perfused blood. An increase in the infusion rate of adenosine made the enhancement more evident, while the 'self-inhibition' of the vasoconstrictor response was more clearly demonstrated. The results were the same with renal nerve stimulation. The vessels respond more effectively to exogenous or endogenous norepinephrine when the renal vascular beds are desensitized to adenosine. These effects disappeared promptly after cessation of adenosine infusion. Thus, the concentration of adenosine at the moment modulates the adrenergic vascular effect in the kidney. These observations might well be important for an understanding of the adrenergic mechanism controlling the renal circulation.

Dipyridamole is a potent coronary vasodilator but a selective constrictor of the renal artery as are adenosine and AMP. It has been reported to decrease the rate of degradation of adenosine and adenine nucleotides (Koss et al. 1963, Bunag et al. 1964), thus potentiating their actions (Hashimoto et al. 1964, Stafford 1966). Recently Sakai et al. (1968) reported that the postocclusive ischemia in the renal artery was potentiated by dipyridamole and blocked by phenoxybenzamine. When postocclusive ischemia was slight, it became marked by alternate treatment with adenosine and norepinephrine. As phenoxybenzamine blocks the vasoconstriction induced by norepinephrine but not that by adenosine, it is suggested that postocclusive vasoconstriction is mainly developed by the renal sympathetic mechanism and that adenosine modulates this through adenosine-norepinephrine interaction. Furthermore, Ono et al. (1966) observed that the lost autoregulation
of renal circulation was restored by dipyridamole, which suggests that dipyridamole as a potentiator of adenine compounds has a favorable effect on the autoregulation of the renal blood flow. From these observations, we assume that the peculiar interaction between adenosine and norepinephrine may contribute to the autoregulation of the renal circulation.

AMP acted in a manner similar to that of adenosine, but seemed to be a little less effective. AMP also induced vasodilation after recovery from the initial decrease in blood flow by infusion in higher dose (1 mg/min). In the potency of inhibiting the response to adenosine, there was statistically no significant difference between infusion of adenosine and AMP, but in potentiating the response to norepinephrine, adenosine was more potent and the difference was statistically significant. Decreases in responses to adenosine at various infusion rates of adenosine showed a similar tendency to those by infusion of AMP, ADP and ATP. The differences in these changes are statistically not significant between those caused by infusion of adenosine and AMP (0.05<p), significant between adenosine and ADP or ATP (p<0.05), and not significant for AMP, ADP and ATP. We cannot make any conclusive statement regarding these differences, because adenine nucleotides are partly hydrolyzed to adenosine in the blood. ADP which induced vasoconstriction only in a small dose was less potent in that effect than adenosine or AMP. ATP which induced only vasodilation showed the least effect, although it has been suggested by some authors that it plays a role in the autoregulation of renal blood flow (Harvey 1964, Scott et al. 1965, Tagawa and Vander 1970).

We must consider whether adenosine or AMP plays a physiological role in the local regulation of renal blood flow. At present, there are no data concerning the rate of generation of adenosine and AMP in the renal tissue by change of perfusion pressure. Gordon (1960) found AMP in the renal venous effluent after release of renal artery occlusion, and this is compatible with findings in bioassay studies of renal venous blood during both autoregulation and reactive hyperemia (Scott et al. 1965). Furthermore, Gerlach et al. (1963) reported that in complete ischemic kidneys, AMP was decomposed only via IMP, bypassing adenosine. They measured the amount of nucleotides in the renal tissue after ischemia and found no accumulation of adenosine. Thurau (1964) assumed a role of AMP in the autoregulation of renal blood flow from the point of view of nucleotide metabolism. Earlier, Conway and Cooke (1939) reported a relatively high amount of adenosine deaminase in renal tissue. Recently, Weidemann et al. (1969) reported that the major fate of AMP added to the perfusion medium in isolated kidney preparations was dephosphorylation to adenosine by 5'-nucleotidase, while the pathway via IMP was a minor one. Thus, adenosine converted from AMP may play an important role in local regulation of renal blood flow.

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References