FEMORAL VASCULAR RESPONSES TO PURINE AND PYRIMIDINE DERIVATIVES: RELEASE OF 5-HYDROXYTRYPTAMINE BY PURINE DERIVATIVES IN ISOLATED, CROSS-CIRCULATED RAT HINDLIMB*

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Abstract—The mode of actions of the purines, adenosine, adenosine-5'-triphosphate (ATP), guanosine and guanosine-5'-triphosphate (GTP), and the pyrimidines, cytidine, cytidine-5'-triphosphate (CTP), thymidine, thymidine-5'-triphosphate (TTP), uridine and uridine-5'-triphosphate (UTP) was investigated in the isolated hindlimb preparation of the rat. A single injection of adenosine, ATP, GTP or UTP into the femoral artery induced a biphasic response, a prominent vasoconstriction preceded by a transient vasodilatation, whereas guanosine and uridine caused only a vasoconstriction. Cytidine, CTP, thymidine and TTP were almost ineffective on the vascular bed. The vasoconstrictor responses to adenosine, ATP, GTP and GTP were effectively antagonized by either methysergide or reserpine, whereas those to uridine and UTP were not modified by either methysergide or phentolamine. Adenine, D-(-)-ribose and hypoxanthine had no effect on the vascular bed. The 5-hydroxytryptamine (5-HT) release from the hindlimb was fluorometrically determined. The present results provide direct evidence that the vasoconstriction caused by ATP, adenosine, GTP and guanosine is attributed to the release of 5-HT from their stores and that purine nucleotides and nucleosides are capable of releasing 5-HT.

In 1880, Gaskell (1) suggested a possible metabolic vasoactive regulation of local blood flow in skeletal muscle. Subsequent studies have served to enhance the attractiveness of this hypothesis and to extend its application to other organs (2-4). The adenosine (3) and the purinergic nerve hypothesis (5-7) proposed later, have renewed interest in the physiological role of purine derivatives in various organs.

Recently, we reported that when inosine, IMP, adenosine and adenine nucleotides were given into the femoral artery of the rat, a prominent vasoconstriction was induced and such was presumably mediated by a tryptaminergic mechanism (8-10). Although several workers have suggested an interaction between purinergic and adrenergic mechanisms in peripheral circulation (11-13), there are few reports indicating the interaction between purinergic and tryptaminergic mechanisms.

The present study was undertaken to determine the mode of actions of the pyrimidine and purine derivatives on the femoral vascular bed of the rat, and an attempt was made to provide direct evidence for the release of 5-hydroxytryptamine (5-HT) by purine derivatives.

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MATERIALS AND METHODS

The isolated hindlimb preparation of the rat has been described previously in detail (9–10) and a brief note is given herein.

Blood-perfused hindlimb

Male albino Sprague-Dawley rats were allowed free access to food and water overnight prior to experiments. Recipient rats (350–400 g) were anesthetized with sodium pentobarbital, 65 mg/kg i.p. and donor rats (550–750 g) with sodium pentobarbital, 70 mg/kg i.p. The right hindlimb of the recipient was completely isolated from the body. The isolated limb was perfused at a fixed flow rate through the femoral artery with heparinized blood (37°C) from the carotid artery of a donor by the aid of a peristaltic pump (Mitsumi Science, SJ-1210). Flow rate was precalibrated and re-checked at the end of the experiment. A square wave electromagnetic flowmeter (Nihon Kohden, MF-25) was used for the measurement of the femoral blood inflow. The blood pressure of the donor and the mean perfusion pressure were continuously measured by means of pressure transducers (Nihon Kohden, MPU-0.5). Recordings were made on an ink-writing rectigraph (TOA Electronics, EPR-3T). The venous outflow from the right femoral vein of the recipient was received in a venous reservoir and in turn returned to the jugular vein of the donor by about a 15 cm drop in hydrostatic pressure.

Hindlimb perfused with Krebs solution

The animals were prepared in the same way as in the blood-perfusion experiments except that no donor was used. The right hindlimb was perfused at a constant flow by means of a peristaltic pump (Mitsumi Science, SJ-1210). The Krebs solution as the perfusate contained the following substances (mM): NaCl 119, KCl 4.8, CaCl₂ 2.5, NaHCO₃ 25, KH₂PO₄ 1.2, MgSO₄ 1.2, glucose 11.0; and was aerated thoroughly with a gas phase mixture containing 95% O₂: 5% CO₂. The oxygen tension of the perfusate (pH 7.4) was always in excess of 600 mm Hg. The temperature was maintained between 36 and 38 °C as it entered the hindlimb. Measurements of PO₂ and pH in the perfusate were carried out using the blood gas analysing system (Radiometer, BMS3-MK2, Copenhagen, Denmark). The 5-HT concentrations in effluent fluid from the perfused limb were determined fluorometrically using a Hitachi Fluorescence Spectrophotometer (MPF-3), as described by Curzon and Green (14).

Reserpine (5 mg/kg) was given s.c. at 48 and 24 hr, but in the experiments with Krebs solution at 72, 48 and 24 hr before experiment.

The following drugs were used: adenosine, adenosine-5'-triphosphate disodium (ATP), guanosine, cytidine, cytidine-5'-triphosphate sodium (CTP), thymidine, thymidine-5'-triphosphate sodium (TTP), uridine and 5-hydroxytryptamine creatine sulfate (Sigma, U.S.A.), guanosine-5'-triphosphate sodium (GTP) (P-L Biochemicals Inc., U.S.A.), uridine-5'-triphosphate sodium (UTP) (Nutritional Biochemicals Corporation, U.S.A.), adenine hydrochloride, D(-)-ribose and hypoxanthine (Tokyo Kasei), (-)-noradrenaline hydrochloride (Sankyo), reserpine (Daiichi Seiyaku), methysergide tartrate (Sandoz, Switzerland).
and phentolamine mesylate (CIBA-Geigy, Switzerland). All the drugs except hypoxanthine were dissolved in 0.9\% saline as stock solutions and were diluted with 0.9\% saline just before use. Hypoxanthine was dissolved in an alkaline solution (pH about 10). In the blood-perfused hindlimb preparations, drug solutions were given into the femoral artery in a volume of 0.01 ml over a period of 4 sec by the use of individual microsyringes (Jintan Terumo Co.). Doses of adenosine, guanosine, cytidine, thymidine, uridine, D-(\textsuperscript{+})-ribose and hypoxanthine refer to their bases and those of the other drugs to their salts, unless otherwise noted. A single intra-arterial injection of 0.9\% saline or alkaline (pH about 10) solution (0.01 ml) in 4 sec had only a slight effect. Increases (vasoconstriction) or decreases (vasodilatation) in the perfusion pressure caused by drugs were taken as drug responses, as the perfusion flow rate was constant.

Values in the text are the means±S.E. Differences between mean values were analysed by Student’s t-test and the P values are given.

RESULTS

Blood-perfused hindlimb

**Basal values of main parameters:** The preparations stabilized within 30 min after the beginning of perfusion. At this stage, the mean perfusion pressure and femoral blood flow were 98.9±0.9 mmHg (N=43) and 3.2±0.1 ml/min, respectively. Stable condition of the preparations was maintained for over 3 hr.

**Dose-response relationships for purine and pyrimidine derivatives in the femoral vascular bed:** Single intra-arterial injections of adenosine (3-30 µg) or ATP (3-30 µg) caused a biphasic response in the femoral vasculature, namely a potent vasoconstriction preceded by a transient vasodilatation. The vasoconstrictor responses to adenosine and ATP were dose-dependent within the dose-range tested. GTP (10-100 µg) and UTP (10-100 µg) induced responses similar to those of adenosine and ATP, but were less effective in inducing the vasoconstrictor response. Guanosine (10-100 µg) and uridine (100-300 µg) were weak vasoconstrictors, while thymidine (100-300 µg), TTP (10-100 µg), cytidine (100-300 µg) and CTP (10-100 µg) were ineffective or had a slight dilatory effect in large doses. Fig. 1A is typical of such experiments and Fig. 1B shows dose-response relations for peak changes in the perfusion pressure after single intra-arterial injections of these substances.

**Effect of methysergide on the vasoconstrictor response to the purines and pyrimidines:** In untreated preparations 5-HT (0.3 µg), noradrenaline (0.1 µg), adenosine (30 µg), ATP (30 µg), guanosine (100 µg), GTP (100 µg), uridine (300 µg) and UTP (100 µg) were given in the randomized order into the femoral artery, respectively, and responses to these substances served as controls. The effect of methysergide was examined in other preparations in which these substances were given in the presence of methysergide (1 µg). The vasoconstriction in response to 5-HT (0.3 µg) but not to noradrenaline (0.1 µg) was antagonized specifically by methysergide (1 µg). The antagonism by methysergide lasted for 30-60 min and this dose of methysergide had almost no effect on the perfusion pressure. The vasoconstrictor effects of adenosine (30 µg), ATP (30 µg), guanosine (100 µg) and GTP (100 µg)
Responses of the femoral vasculature to increasing doses of adenine, guanine, cytidine, thymidine and uridine derivatives injected into the femoral artery.

A) Original recordings. PP, perfusion pressure.

B) Dose-response relationships. Hatched and open columns refer to vasodilator and vasoconstrictor components of responses (mm Hg), respectively. Vertical bars represent means ± S.E. and the numbers of experiments are given in parentheses.

Fig. 1. Responses of the femoral vasculature to increasing doses of adenine, guanine, cytidine, thymidine and uridine derivatives injected into the femoral artery. A) Original recordings. PP, perfusion pressure. B) Dose-response relationships. Hatched and open columns refer to vasodilator and vasoconstrictor components of responses (mm Hg), respectively. Vertical bars represent means ± S.E. and the numbers of experiments are given in parentheses.
were antagonized significantly by the same dose of methysergide, whereas the effects of uridine (300 μg) and UTP (100 μg) were not modified by methysergide. Summarized data are shown in Fig. 2.

**Effect of phentolamine on the vasoconstrictor response to uridine and UTP:** A single injection of 10 μg of phentolamine into the femoral artery slightly decreased the perfusion pressure. This dose of phentolamine significantly reduced the vasoconstrictor response to noradrenaline (0.1 μg). The antagonism by phentolamine lasted for over 30 min. The vasoconstriction in response to uridine (300 μg) and UTP (100 μg) was not modified by the same dose of phentolamine. Summarized data are shown in Fig. 3.

**Vascular responses to adenosine, ATP, guanosine and GTP in reserpine-treated animals:** A single dose of either adenosine (30 μg), ATP (30 μg), guanosine (100 μg) or GTP (100 μg) was injected into the femoral artery of reserpine-treated preparations. As shown in Fig. 4, the vasoconstrictor responses to these substances were significantly less compared with those in untreated preparations.

**Responses to adenine, D(-)-ribose and hypoxanthine on the femoral vascular bed:** A single injection of either adenine (100 μg), D(-)-ribose (100 μg) or hypoxanthine (100 μg) was given into the femoral artery. These substances had almost no effect on the vascular bed. Fig. 5 illustrates the typical results from 2–3 experiments.

**Hindlimb perfused with Krebs solution**

**5-HT release by ATP, adenosine, GTP and guanosine from the hindlimb:** The hindlimb...
preparations perfused with the constant pumping volume of Krebs solution became stable about 10 min after the onset of perfusion. A single injection of either ATP (1 mg), adenosine (1 mg), GTP (2 mg) or guanosine (2 mg) into the femoral artery was principally given to a single preparation. In untreated preparations, the induced vasoconstriction, which lasted longer than 5 min, reached a maximum about 30 sec after the injection of these substances. Then, the effluent fluid from the femoral vein was collected during 30 sec and the 5-HT concentrations were determined. In untreated preparations, single injections of ATP, adenosine, GTP and guanosine increased the output of 5-HT in the effluent fluid from an undetectable amount to about 0.3 μg/min, but in reserpine-treated ones appreciable increase of 5-HT output with these substances was not observed. As in reserpine-treated preparations the weak vasoconstrictor effects of these substances wore off within 60 sec, the effluent fluid was collected during 30 sec immediately after the administration of these substances. UTP (2 mg) and uridine (2 mg) did not induce a release of 5-HT from the hindlimb (not shown). Table 1 shows the vasoconstrictor responses to ATP, adenosine, GTP and guanosine, and an appreciable amount of 5-HT released when these substances were injected.

Fig. 3. Effect of phentolamine on the vasoconstriction in response to noradrenaline (NA), uridine (Urd) and UTP. Open columns: untreated; hatched columns: treated with phentolamine (10 μg). Other details as in Fig. 1.

Fig. 4. Vasoconstrictor responses of the femoral vascular bed to the purines and tyramine (Tyr) in untreated (open columns) and reserpine-treated (hatched columns) preparations. Other details as in Fig. 1.

Fig. 5. Responses of the femoral vascular bed to adenine, D-(-)-ribose and hypoxanthine. Original recordings from 2-3 experiments are illustrated.
TABLE 1. 5-Hydroxytryptamine (5-HT) release by ATP, adenosine, GTP and guanosine from the perfused hindlimb

| Drugs      | Max. J increase in perfusion pressure (mmHg) | Increase of 5-HT output† (μg/min) |
|------------|---------------------------------------------|----------------------------------|
|            | Untreated | Treated   | Untreated | Treated   |
| ATP 1 mg   | 59.4 ± 8.7 | 8.3 ± 10.9* | 0.303 ± 0.049 | undetectable |
| Adenosine 1 mg | 71.5 ± 9.8 | 14.3 ± 7.0** | 0.350 ± 0.047 | undetectable |
| GTP 2 mg   | 61.0 ± 9.0 | 9.0 ± 1.0** | 0.308 ± 0.051 | undetectable |
| Guanosine 2 mg | 107.3 ± 13.0 | 4.6 ± 2.5*** | 0.328 ± 0.067 | undetectable |
| 5-HT 1 μg  | 54.3 ± 5.9 | 70.3 ± 13.6 | 0.244 ± 0.084 |              |

† Differences between values before and after drug administrations. Values are expressed as mean ± S.E., numbers of experiments are shown in parentheses. P versus untreated groups: *: < 0.02, **: < 0.01, ***: < 0.001. All values for 5-HT and the doses of drugs refer to the base. Initial perfusion pressure: untreated, 41.2 ± 1.7 mmHg (N=16); reserpine-treated, 37.8 ± 3.4 mmHg (N=13), P>0.05. Perfusion flow rate, 5.5 ml/min.

DISCUSSION

The present experiments show that the purines and pyrimidines tested have characteristic vascular effects on the femoral vascular bed of the rat when given intra-arterially. Other workers have examined the mode of actions of adenine, uridine and cytidine derivatives on the femoral vasculature of dog, however, they did not detect a definite vasoconstrictor response to purine compounds (15-17).

We found that a potent vasoconstriction produced after the injection of adenosine or inosine into the femoral artery of the rat was abolished by methysergide or reserpine (8-9). Further investigation demonstrated that ATP, ADP, AMP and IMP, like adenosine and inosine, had remarkably similar pharmacological features (10).

According to the present study, the vasoconstriction in response to guanosine and GTP as well as adenosine and ATP was blocked significantly by methysergide or reserpine. In parallel with the previous results (8-10), it seems likely that the purines induce a vasoconstriction in the rat femoral vasculature through a common mechanism, presumably by 5-HT released from the peripheral storage sites. This suggestion is strongly supported by direct evidence that ATP, adenosine, GTP and guanosine injected into the femoral artery released an appreciable amount of 5-HT into the effluent fluid from the perfused hindlimb. The fact that intra-arterial injections of adenine, D(-)-ribose and hypoxanthine had no effect on the vasculature suggests that the conjugation of the purine base, adenine, hypoxanthine or guanine with a ribose moiety is a necessity in the development of activity releasing 5-HT in the rat hindlimb.

On the other hand, the vasoconstriction after uridine and UTP was not modified by
treatment with either methysergide or phentolamine. Moreover, these substances did not induce a release of 5-HT from the perfused hindlimb. The vasoconstriction caused by these substances, therefore, seems unlikely to be mediated by either tryptaminergic or adrenergic mechanisms. The mechanism involved in response to cytidine, CTP, thymidine and TTP was not elucidated in this study, since these substances were almost ineffective on the femoral vasculature. Thus, the tryptaminergic vasoconstrictor action seems to be restricted to the substituted purine nucleotides and nucleosides. Elucidation of a physiological role of interaction between the purines and 5-HT in other organs or different species remains the subject of further investigation.

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REFERENCES

1) Gaskell, T.W.H.: On the tonicity of the heart and blood vessels. J. Physiol. 3, 48–75 (1880)
2) Barcroft, H.: Circulation in skeletal muscle. Handbook of Physiology, Circulation, Edited by Hamilton, W.F. and Dow, P., Sect. 2, Vol. II, p. 1353, Am. Physiol. Soc., Washington, D.C. (1963)
3) Berne, R.M.: Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. Am. J. Physiol. 204, 317–322 (1963)
4) Haddy, F.J. and Scott, J.B.: Metabolically linked vasoactive chemicals in local regulation of blood flow. Physiol. Rev. 48, 688–707 (1968)
5) Burnstock, G.: Purinergic nerves. Pharmacol. Rev. 24, 509–581 (1972)
6) Burnstock, G., Campbell, G., Satchell, D.G. and Smythe, A.: Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. Brit. J. Pharmacol. 40, 668–688 (1970)
7) Burnstock, G., Satchell, D.G. and Smythe, A.: A comparison of the excitatory and inhibitory effects of non-adrenergic, non-cholinergic nerve stimulation and exogenously applied ATP on a variety of smooth muscle preparations from different vertebrate species. Brit. J. Pharmacol. 46, 234–242 (1972)
8) Sakai, K. and Akima, M.: Tryptaminergic vasoconstriction induced by adenosine in the femoral vascular bed of rat. Japan. J. Pharmacol. 27, 908–910 (1977)
9) Sakai, K. and Akima, M.: Vasoconstriction after adenosine and inosine in the rat isolated hindlimb abolished by blockade of tryptaminergic mechanisms. Arch. Pharmacol. 302, 55–59 (1978)
10) Sakai, K.: Tryptaminergic mechanism participating in induction of vasoconstriction by adenine nucleotides, adenosine, IMP and inosine in the isolated and blood-perfused hindlimb preparation of the rat. Japan. J. Pharmacol. 28, 579–587 (1978)
11) Sakai, K., Yasuda, K. and Hashimoto, K.: Role of catecholamine and adenosine in the ischemic response following release of a renal artery occlusion. Japan. J. Physiol. 18, 673–685 (1968)
12) Buckley, N.M.: Cardiac effects of nucleosides after propranolol treatment of isolated hearts. Am. J. Physiol. 218, 1399–1405 (1970)
13) Hashimoto, K. and Kokubun, H.: Interaction between adenine compounds and norepinephrine in dog renal circulation. Tohoku J. Exp. Med. 107, 373–380 (1972)
14) Curzon, G. and Green, A.R.: Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. Brit. J. Pharmacol. 39, 653–655 (1970)
15) Hashimoto, K., Kumakura, S. and Tanemura, I.: Mode of action of adenine, uridine and cytidine nucleotides and 2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido-(5,4-d) pyrimidine on the coronary, renal and femoral arteries. Arzneim.-Forsch. 14, 1252–1254 (1964)
16) Hashimoto, K. and Kumakura, S.: The pharmacological features of the coronary, renal, mesenteric and femoral arteries. *Japan. J. Physiol.* **15**, 540–551 (1965)

17) Sakai, K., Sugano, S., Taira, N. and Hashimoto, K.: Pharmacological features of peripheral vascular beds of beagles. *Japan. J. Pharmacol.* **24**, 659–669 (1974)