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Naturally occurring 2′-*O*-methylpurine nucleosides with hypotensive properties

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Abstract. 2′-*O*-Methylinosine (**1**) has been isolated for the first time and shown to be an intrinsic hypotensive principle. Its probable in vivo precursor, 2′-*O*-methyladenosine (**3**), showed stronger and even orally potent hypotensive activity. Resistance of the methyladenosine (**3**) against adenosine deaminase is thought to contribute to its long-lasting activity. The effect of both nucleosides (**1** and **3**) was not accompanied with any significant change in heart rate, which is often observed with adenosine.

Key words. Methyladenosine; methylinosine; hypotension; adenosine; adenosine deaminase.

In the course of a search for novel low molecular weight bioactive metabolites induced by inflammation in animals, we previously isolated several bioactive heterocyclic compounds from extracts of inflamed rabbit skin tissues [1, 2]. In seeking compounds with a hypotensive effect on spontaneously hypertensive rats (SHR), we fractionatd the phenol extract [1] of the inflamed rabbit skin tissues inoculated with vaccinia virus and obtained 2′-*O*-methylinosine (**1**) as one of the active principles. In this paper, we report that both (**1**) and its precursor, 2′-*O*-methyladenosine (**3**), have unique hypotensive activities.

Materials and methods

Isolation of 2′-*O*-methylinosine (1). In the following fractionation, hypotensive effect on SHR was used as an index to obtain an active component. After boiling the aqueous phenol extract of inflamed rabbit skin tissues [1], and evaporation, the resulting residue was first applied onto a Bio-Gel P-2 column (H_2O), and active fraction was separated by high-performance liquid chromatography (HPLC) column (ODS, 0.1% trifluoro-acetic acid (TFA)-MeOH) to give a colourless crystalline compound as an active principle, 2′-*O*-methylinosine (**1**), melting point (mp) 180–181 °C; ^{1}H NMR (400 MHz, D_2O) δ 3.39 (3H, s), 3.85 (1H, dd, $J = 3.42$, 12.69), 3.94 (1H, dd, $J = 2.93$, 12.69), 4.30 (1H, m), 4.52 (1H, t, $J = 5.85$), 4.61 (1H, t, $J = 5.85$), 6.19 (1H, d, $J = 5.85$), 8.25 (1H, s), 8.39 (1H, s); SIMS m/z 283 (MH^+). Its structure was determined by comparison of its physical data with those of a synthetic authentic specimen. Results of co-melting and co-elution on HPLC of both isolated and synthetic specimens showed that they are identical; HPLC conditions were as follows: system, JASCO 800 series (JASCO, Japan); column, inertsil ODS-2 (4.6×105 mm, GL Science, Japan); column temp., room temp.; eluent, 10% MeOH containing 0.1% TFA; flow rate, 0.8 ml/min; detection, ultraviolet (UV, 250 nm) with 875-UV (JASCO, Japan); retention time of the single peak of 2′-*O*-methylinosine, 8 min after injection.

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Table 1. Effect of 2′-*O*-methylinosine and α-methyldopa on systolic blood pressure on SHR.

Drug	Dose (mg/kg, i.p)	No. of rats	Blood pressure (mmHg)		% Decrease
			before adm.	2 h after adm.	
Control	-	3	233 ± 7	231 ± 7	0.4 ± 1.8
2′-*O*-Me Ino	100	3	245 ± 8	97 ± 7***	60.4 ± 3.5^{+++}
α-Methyldopa	100	3	242 ± 5	185 ± 12*	24.8 ± 5.8^{+}

Each value represents the mean value ± SE.
***,* Significantly different from control at $P < 0.001$ and $P < 0.05$, respectively.
$^{+++}$,$^{+}$ Significantly different from the value observed before administration at $P < 0.001$ and $P < 0.05$, respectively (t test).

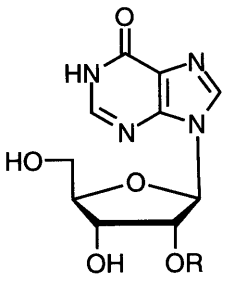
Preparation of 2′-*O*-methyladenosine (3). A modified method of Robins et al. was adapted [3]. A suspension of adenosine (**4**) (37.5 nmol) in a solution of $SnCl_2$ (2 mmol) in MeOH (2 l) was treated with excess diazomethane (200 mmol) in 1,2-dimethoxyethane. The solvents were removed in vacuo, and the residue was chromatographed on Dowex 1 × 8 (OH^- form) with 60% EtOH as an eluent. The appropriate fractions were collected and evaporated to dryness, and the residue was recrystallized from EtOH to give pure 2′-*O*-methyladenosine (**3**) in 34% yield; mp 205–206 °C (lit., 200–202 °C [3]) (Found: C, 47.0; H, 5.4; N, 24.7. $C_{11}H_{13}N_5O_4$ requires C, 47.0; H, 5.4; N, 24.9); ^{1}H NMR (400 MHz, D_2O) δ 3.39 (3H, s), 3.86 (1H, dd, $J = 2.90$, 12.70), 3.93 (1H, dd, $J = 2.40$, 12.70), 4.26 (1H, m), 4.40 (1H, t, $J = 5.90$), 4.56 (1H, dd, $J = 5.90$, 4.90), 6.01 (1H, d, $J = 5.90$), 8.01 (1H, s), 8.20 (1H, s); SIMS m/z 282 (MH^+).

Preparation of 2′-*O*-methylinosine from 2′-*O*-methyladenosine. Sodium nitrite (72 mmol) was added to a solution of **3** (3.6 mmol) in 25% aqueous acetic acid (10 ml) maintained at 20 °C. After 5 h, the reaction mixture was neutralized with solid sodium bicarbonate and applied to an Amberlite XAD-7 column. The column was washed with water and eluted with 30% MeOH. The eluate was evaporated to dryness, and the residue was recrystallized from MeOH to give pure 2′-*O*-methylinosine (**1**) in 72% yield; mp 180–181 °C (lit., 177–180 °C [3]) (Found: C, 47.1; H, 5.1; N, 19.6. $C_{11}H_{14}N_4O_5$ requires C, 46.8; H, 5.0; N, 19.9); ^{1}H NMR (400 MHz, D_2O) δ 3.40 (3H, s), 3.81 (1H, dd, $J = 3.42$, 12.70), 3.94 (1H, dd, $J = 2.93$, 12.70), 4.30 (1H, m), 4.52 (1H, t, $J = 5.85$), 4.61 (1H, t, $J = 5.85$), 6.19 (1H, d, $J = 5.85$), 8.29 (1H, s), 8.42 (1H, s); SIMS m/z 283 (MH^+).

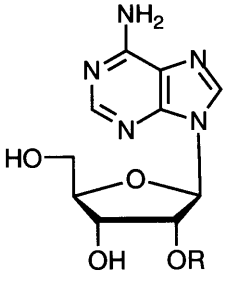
Experimental animals. Male spontaneously hypertensive rats (SHR; 16 weeks old, Hoshino-Dohbutu, Japan) were kept at 22–24 °C, 45–70% humidity, and were fasted for 18 h before the experiment.

Measurement of systolic blood pressure. Systolic blood pressure was measured by an indirect method using a pressure cuff and ring-shaped pulse transducer (Narco-Bio-Systems, Houston, TX, USA, PE-300). Pressure changes and pulse waves were recorded before and 2 h after administration of a sample, and heart rate was determined from the pulse trace at the same time. Each effect was compared with those of a saline control and α-methyldopa as placebo. In each case, the difference in pressure before and 2 h after administration was calculated as percentage decrease.

Adenosine deaminase assay. The stability of adenosine (**4**) or 2′-*O*-methyladenosine (**3**) in the presence of adenosine deaminase (EC 3.5.4.4.) was followed by measuring the change in optical density at 265 nm resulting from the conversion of **4** to **2**, or **3** to **1** using a spectrophotometer (Beckman, model DU-650, Fullerton, CA, USA). The assay mixture in 2 ml contained 50 mM phosphate buffer (pH 7.5), 0.1 mM test sample, and 0.02 U of adenosine deaminase at 25 °C. Deaminase inhibition was determined by observing the decrease in the rate of adenosine deamination.



1 :R=Me
2 :R=H



3 :R=Me
4 :R=H

Results and discussion

Fractionation of the extract of inflamed rabbit skin tissues provided a colourless crystalline compound as one of the hypotensive principles. This was identified as 2′-*O*-methylinosine (**1**); its presence in vivo had not been previously demonstrated. 2′-*O*-Methylinosine (**1**) exhibited a significant hypotensive effect in decreasing blood pressure by about 40% when administered by intraperitoneal (i.p.) injection at a dosage of 100 mg/kg, and showed stronger activity than α-methyldopa at the

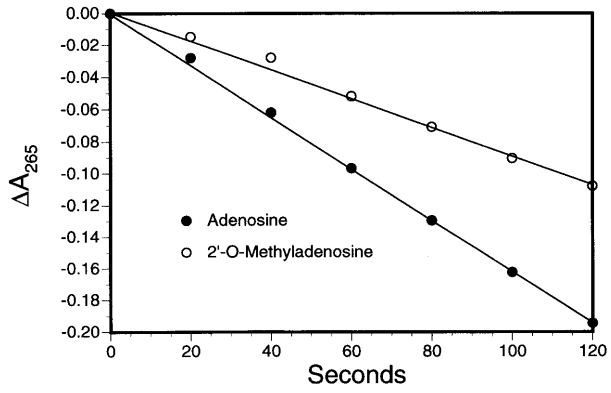


Figure 1. Stability of adenosine and 2′-*O*-methyladenosine against adenosine deaminase.

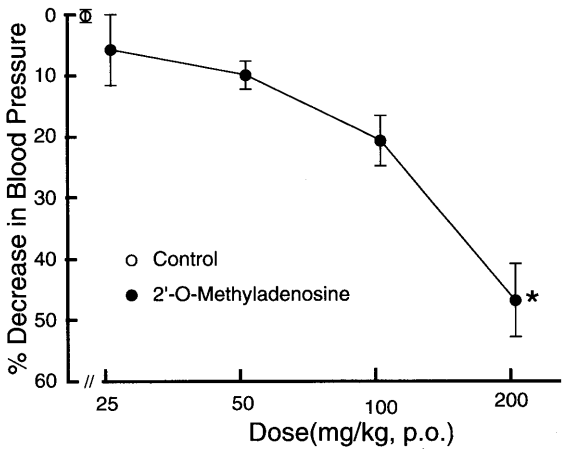


Figure 2. Dose-hypotensive effects of 2′-*O*-methyladenosine on systolic blood pressure in SHR. Each point and vertical bar represent the mean ± SE. *Significantly different from control at $P < 0.05$.

same dosage (table 1). This nucleoside (**1**) was thought most likely to be a metabolite of 2′-*O*-methyladenosine (**3**), known as a component in RNA [4]. In fact, we have found that **3** can be enzymically converted to **1** by adenosine deaminase (see fig. 1), but its rate is much slower than that for the conversion of adenosine (**4**) to inosine (**2**).

Although adenosine (**4**) itself had been known to have hypotensive effects, *O*-methylated adenosine (**3**) had not previously been shown to have similar effects, nor would they have been possible to predict. It had been widely believed that the free 2′-OH group in the ribose moiety of adenosine was necessary for its hypotensive activity [5], and that *O*-methylation at the 2′-position would cause inactivation. On the other hand, adenosine (**4**) had been known to show much stronger hypotensive activity than inosine (**2**) [6], and if 2′-*O*-methylinosine (**1**) was hypotensive, then 2′-*O*-methyladenosine (**3**) could be analogously hypotensive. In order to clarify these points, we compared the hypotensive activities of these two naturally occurring methylated purine nucleosides (**1** and **3**) with each other and with their unmethylated nucleosides (**2** and **4**).

All four natural nucleosides (**1**–**4**) showed more or less hypotensive activities when they were injected via the i.p. route (data not shown). However, as shown in table 2, neither methylinosine (**1**), inosine (**2**) nor adenosine (**4**) showed hypotensive activity when administered orally at dosages of 200 mg/kg. On the contrary, 2′-*O*-methyladenosine (**3**) decreased blood pressure significantly in a dose-dependent manner (fig. 2) 2 h after oral administration, and this effect was more prominent than with α-methyldopa under the same conditions. Far from inactivation, our result indicated that 2′-*O*-methylation enhanced the original activity of (**1**). Furthermore, the hypotensive effects of both natural methylpurine nucleosides (**1** and **3**), by i.p. injection of **1** and i.p. or administration per os (p.o.) of **3**, was not accompanied with any significant change in heart rate.

The hypotensive action of adenosine (**4**) occurs through several mechanisms, and it includes vasodilatation in the peripheral vasculature and cardiac depression: the former is probably mediated by A_2 receptors and the latter by A_1 receptors [5]. However, extrinsic **4** is so short-lasting that it could not be orally active owing to

Table 2. Effect of 2′-*O*-methyladenosine, -inosine and α-methyldopa on systolic blood pressure on SHR.

Drugs	Dose (mg/kg, p.o.)	No. of rats	Blood pressure (mmHg)		% Decrease
			before adm.	2 h after adm.	
Control	-	3	227 ± 8	236 ± 11	−4.0 ± 1.8
Adenosine	200	3	191 ± 9	202 ± 12	−6.1 ± 2.6
Inosine	200	3	193 ± 7	200 ± 5	−3.7 ± 1.2
2′-*O*-Me Ado	200	3	218 ± 21	103 ± 21*	53.2 ± 7.3^{+}
2′-*O*-Me Ino	200	3	239 ± 6	210 ± 12	12.0 ± 2.7
α-Methyldopa	200	3	235 ± 14	186 ± 6	22.0 ± 2.5^{++}

Each value represents the mean value ± SE.
* Significantly different from control at $P < 0.05$.
$^{+}$ Significantly different from the value observed before administration at $P < 0.05$ (*t* test).

its rapid uptake into cells, deamination by adenosine deaminase and/or phosphorylation by adenosine kinase. The reason 2′-*O*-methyladenosine (**3**) was orally hypotensive is not yet clear. Some stable A_2-selective adenosine agonists (e.g. CGS 21680) showed hypotensive effects without affecting heart rate [7]. So there is a possibility that **3** may act as an A_2-selective adenosine agonist. However, because the free 2′-OH group in the ribose moiety of adenosine has been reported to be essential for the manifestation of receptor affinity [5], we are now evaluating the interaction of **3** with receptor(s), although this possibility appears to be doubtful at present. Resistance of methyladenosine (**3**) against adenosine deaminase may well be partly responsible for its potent and long-lasting activity, since methyladenosine (**3**) was more stable to the action of adenosine deaminase than adenosine, as shown in figure 1. As an alternative possibility, the potentiation of endogenous adenosine by methyladenosine (**3**) may play a more important role in its potent and long-lasting activity. Methyladenosine (**3**) was a competitive inhibitor of adenosine deaminase, with K_i values of 1.3×10^{-5} M. The mechanism of this inhibition will be the subject of a future paper.

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