Jpn. J. Pharmacol. 78, 345–354 (1998)

Effects of Adrenergic and Nitrergic Blockade on Theophylline-Induced Increase in Peripheral Blood Flow in Rat Ear

Fujiko Sanae and Hisao Hayashi

Department of Medicine, Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3 Kanagawa, Kanazawa 920–1181, Japan

Received April 28, 1998 *Accepted August 26, 1998*

ABSTRACT—A bolus injection of theophylline produced a significant increase in peripheral blood flow in anesthetized rat ear, monitored by laser-Doppler flowmetry, with increases in arterial blood pressure and heart rate. These effects were attenuated by previous treatment with reserpine, but reserpine had no effect on the blood flow increase produced by acetylcholine. A dose of propranolol, which caused attenuation of the theophylline-induced increase in heart rate, did not change the peripheral blood flow. The higher dose of propranolol, which nearly canceled the increases in blood pressure and heart rate, caused attenuation of the blood flow increase but did not cancel it. However, the theophylline-induced flow increase was completely reversed by a nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester, which alone had no effect, without any change in arterial blood pressure and heart rate. Treatment of the rats with the dose of inhibitor slightly and significantly reduced the response of peripheral blood flow to acetylcholine. The other isomer, N^G-nitro-D-arginine methyl ester, and the other inhibitor, N^G-monomethyl-L-arginine, did not have such an effect. These results suggest that the flow increase is due to an independent effect on the heart with modification by autonomic reflexes and involves the adrenergic and nitrergic pathways.

Keywords: Theophylline, Peripheral blood flow, Reserpine, Propranolol, Nitric oxide synthase inhibitor

Theophylline (1,3-dimethylxanthine) is a non-selective phosphodiesterase inhibitor, although the importance of this property at therapeutic doses remains controversial. At these levels, its cardiovascular effects may be mediated by its ability to block myocardial and vascular adenosine receptors (1). However, methylxanthines without adenosine-antagonistic properties can produce tachycardia (2). Theophylline-induced hemodynamic effects previously described include tachycardia, vasodilation, and positive inotropism, but wide variation occurs between individuals (3). In any case, theophylline has both positive inotropic and chronotropic effects on the heart, while peripheral vascular resistance is reduced, regardless of any change in arterial blood pressure.

The vasorelaxant properties of methylxanthines are generally believed to be mediated by inhibition of phosphodiesterase activity in vascular smooth muscle cells, resulting in increased cellular levels of cyclic AMP (and cyclic GMP) (4), although the activity of theophylline on phosphodiesterase inhibition is comparatively small. Mechanisms other than those involving phosphodiesterase inhibition might be contributing to the theophylline-induced vasodilation in vivo. The endothelium is important in maintaining vascular tone (5), and recent studies have shown that endothelium-dependent vasodilation of isolated rat aorta is induced by methylxanthines such as pentoxifylline and others (6–8). Endothelium-derived relaxing factor is a physiological vasodilator thought to be the nitric oxide radical (NO) (9) or a closely related molecule, perhaps the nitroxyl radical (10) or both. NO is synthesized from its precursor L-arginine in a reaction catalyzed by NO synthase of either the constitutive or inducible form. It activates guanylate cyclase, accelerating formation of cyclic GMP in vascular smooth muscle cells, thus causing relaxation and vasodilation (9). Endogenous NO also modulates adrenergic neural vasoconstriction (11). However, whether NO contributes to theophylline-induced vasodilation is unknown. It has been proposed that theophylline induced endothelium-independent relaxation in rat isolated aorta (6).

In this study, we investigated the effects of theophylline by doing hemodynamic experiments in anesthetized rats, including laser-Doppler flowmetry in the cutaneous vessels of the ear.

MATERIALS AND METHODS

Animals

Male Wistar rats (Nihon SLC, Inc., Hamamatsu) weighing 200−250 g were used in this study. Rats treated with or without reserpine (Apoplon®; Daiichi Pharmaceutical, Osaka) (5 mg/kg, i.p.) 24 hr before the experiments were anesthetized with urethane (800 mg/kg, i.p., supplemented as required) and α-chloralose (80 mg/kg, i.p.) and placed in a supine position on a small animal operating table in a compact space (700 × 500 × 500 mm) to control the internal temperature, which was maintained at 26−28°C with a heating lamp. Rectal temperature was maintained at 37−38°C with a isothermal pad (RBC, Inc., Nagoya) and the lamp.

Materials

The following compounds were used: theophylline (Wako Pure Chemicals, Osaka); acetylcholine chloride (Daiichi Pharmaceutical, Osaka); propranolol hydrochloride and atropine sulfate (Sigma Chemical, St. Louis, MO, USA); and N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-D-arginine methyl ester (D-NAME) and N^G-monomethyl-L-arginine (L-NMMA) (Research Biochemicals International, Natick, MA, USA).

Measurements of hemodynamics

The left carotid artery and vein were catheterized for blood pressure measurement and intravenous injections, respectively. Blood pressure and heart rate were monitored continuously with a pressure transducer (MP5200; Baxter, Tokyo) and recorded on a recorder (WR3701; Graphtec, Yokohama). Heart rate was counted with a cardiotachometer (AT-601G; Nihon Kohden Co., Tokyo) triggered by blood pressure pulses. Peripheral blood flow of the rat's ear was continuously monitored by laser-Doppler flowmetry (12, 13), using a laser-Doppler flowmeter LBF-221 (Biochemical Science Co., Kanazawa). A plate-type probe for laser-Doppler flowmetry was placed with double-sided adhesive tape on the invisible area of blood vessels of the right ear from behind. The placement of a probe that was able to hold to the laser light through the ear from the opposite side was ascertained, located 4−5 mm proximal to the tip of the ear. The conventional flow probe evaluates flow in a 1-mm diameter area. The peripheral blood flow was calculated as the average perfusion recorded during a period of 5 sec. Since the values monitored on the LBF-221 are expressed in dimensionless blood flow values (arbitrary units), changes in the peripheral blood flow measured by laser-Doppler flowmetry were expressed as percentages of the baseline values.

Experimental procedure

The experimental procedure started after a 30-min resting period for stabilization of blood pressure, heart rate, body temperature and cutaneous blood flow. Before experiments, the responses of blood flow to acetylcholine (1 μg/ml, i.v.) were examined. A bolus injection of acetylcholine induced a transient increase in peripheral blood flow with a transient decrease in blood pressure. When the increasing response was not clear, the placement of the flow probe was changed. After an equilibration time, rats were given i.v. bolus injections of each substance, which was dissolved in saline and injected in 1 ml/kg volume. No effects of the saline volume on the hemodynamics were observed in each experiment.

Effects of a single bolus of theophylline on control and reserpinized rats: Theophylline was administered intravenously as a bolus of 1, 3, 5 and 10 mg/kg to control rats (n=5−7). Hemodynamic measurements were recorded 60 min after each dose. To reserpinized rats (n=5) was administered intravenously 5 mg/kg theophylline.

Effects of propranolol and atropine, in the absence and presence of theophylline: Propranolol was administered initially at a dose of 0.5 mg/kg and then 1.0 mg/kg at 5 min interval to control rats (n=5) and theophylline-treated rats (n=7). Atropine (1.0 mg/kg) was administered to control rats (n=4) and theophylline-treated rats (n=4). Theophylline (5 mg/kg, i.v.) was administered 15 min before the injection of propranolol or atropine. Hemodynamic measurements were recorded 10 min after propranolol or atropine.

Effects of L-NAME, D-NAME and L-NMMA in the presence of theophylline: L-NAME (0.3 mg/kg, i.v.) (n=7), D-NAME (0.5 mg/kg, i.v.) (n=4), L-NMMA (1.0 mg/kg, i.v.) (n=5) or saline (n=7) was administered 15 min after the injection of theophylline (5 mg/kg, i.v.). Hemodynamic measurements were recorded 20 min after each arginine analogue.

Effects of L-NAME on response of peripheral blood flow to acetylcholine: The response of blood flow to acetylcholine (1 μg/ml, i.v.) was examined (1st response). Fifteen minutes later, the rat was treated with saline (n=5) or L-NAME (0.3 mg/kg, i.v.) (n=5). The 2nd response to acetylcholine was examined 15 min after the saline or L-NAME. Changes in response to acetylcholine in blood flow were expressed as percentages of the 1st blood flow responses.

Effects of atropine and propranolol, L-NAME, and L-NMMA on response of peripheral blood flow to theophylline: Saline (n=7), atropine (1.0 mgkg) followed by propranolol (1.0 mg/kg) at 7 min (n=5), L-NAME (0.3 mg/kg) (n=5) or L-NMMA (1.0 mg/kg) (n=5) was administered i.v. to rat 15 min before injection of theophylline (5 mg/kg, i.v.). Hemodynamic measurements were

recorded just before and 15 min after theophylline.

Statistical analyses

Results are expressed as the mean±S.E.M. of mean arterial blood pressure, heart rate, and change in peripheral blood flow expressed as percentages of the baseline values of the given number (n) of experiments. The statistical analysis of difference was done by Student's *t*-test or Welch's *t*-test for comparisons between two groups and Dunnett's test for multiple comparisons. Differences were accepted as statistically significant at P values <0.05.

RESULTS

Effects of a single bolus of theophylline on control and reserpinized rats

The mean arterial blood pressure of anesthetized rats was 92.3±1.5 mmHg (n=25), with a heart rate of 395±7 beats/min. Administration of saline as a bolus caused little change in blood pressure, heart rate or peripheral blood flow of anesthetized rats for at least 60 min. Bolus injections of theophylline at doses of 1, 3, 5 and 10 mg/kg, i.v. induced increases in systolic, mean and diastolic blood pressure in a dose-dependent manner. The increase in mean arterial blood pressure was accompanied by an initial transient decrease and reached a plateau within 5 min. Heart rate was also increased by theophylline in a dose-dependent manner. An increase in heart rate was immediately observed and reached a plateau within 5 min. Each dose of theophylline resulted in increases in mean arterial blood pressure and heart rate 15 min after injections from values of those at 0 min: 8.6±1.9 mmHg and 28±5 beats/min in the 1 mg/kg group, 14.6±2.1 and 48±7 beats/min in the 3 mg/kg group, 23.9±2.7 mmHg and 68±7 beats/min in the 5 mg/kg group, and 29.6±5.3 mmHg and 109±13 beats/min in the 10 mg/kg group. The increases in mean arterial blood pressure and heart rate remained for at least 60 min in rats injected with high doses of theophylline (5 and 10 mg/kg), although mean arterial blood pressure and heart rate of rats induced by low doses of theophylline (1 and 3 mg/kg) were slowly reduced. Concerning peripheral blood flow, although injection of 1 mg/kg theophylline caused little change in peripheral blood flow, high doses of theophylline (3, 5 and 10 mg/kg) were frequently accompanied by initial large increases and produced increases in peripheral blood flow of 152±11%, 157±6% and 212±22%, respectively, of the initial level 15 min after injections. The increased levels in peripheral blood flow were slowly reduced 30 min after injections, but the peripheral blood flow influenced by 5 mg/kg remained at the high level of 132±7% after 60 min. The hemodynamic values before, 15 min and 60 min after injection of theophylline (0, 1, 3, 5 and 10 mg/kg, i.v.) and the changes at 15 min and 60 min from values before injection of theophylline are shown in Table 1.

In rats treated with reserpine, the mean arterial blood

Table 1. Effects of i.v. bolus injection of theophylline on mean arterial blood pressure (MBP), heart rate (HR) and peripheral blood flow in ear (PBF) in anesthetized rats

Theophylline (mg/kg)	Time after bolus of theophylline								
	before			15 min			60 min		
	MBP (mmHg)	HR (beats/min)	PBF[a] (%)	MBP (ΔChange) (mmHg)	HR (ΔChange) (beats/min)	PBF[a] (%)	MBP (ΔChange) (mmHg)	HR (ΔChange) (beats/min)	PBF[a] (%)
Saline (n=3)	91.7±1.7	410±17	100	92.7±1.5 (Δ1.0±1.0)	410±17 (Δ0±0)	100±2	92.7±1.5 (Δ1.0±1.0)	417±13 (Δ7±3)	99±2
1 (n=5)	93.0±2.5	400±14	100	101.6±1.9* (Δ8.6±1.9)	430±11 (Δ28±5)	107±3	93.6±4.2 (Δ0.6±2.2)	395±14 (Δ−5±8)	95±5
3 (n=5)	94.0±3.7	399±12	100	108.6±2.2** (Δ14.6±2.1)	447±10 (Δ48±7)	152±11*	105.0±3.8 (Δ13.3±3.7)	429±10 (Δ33±9)	141±14*
5 (n=7)	91.4±2.4	384±9	100	115.3±1.4** (Δ23.9±2.7)	453±8* (Δ68±7)	157±6**	118.6±2.1** (Δ28.6±2.1)	457±10* (Δ72±9)	132±8**
10 (n=5)	91.0±2.4	394±13	100	120.6±3.2** (Δ29.6±5.3)	503±16* (Δ109±13)	212±22*	124.6±1.3** (Δ33.6±3.4)	492±18* (Δ98±15)	137±10*

[a]PBF were expressed as percentages of the baseline values. Values are expressed as means±S.E.M. *P<0.05, **P<0.005 versus saline control.

pressure of anesthetized rats was 75.0±3.2 mmHg, with a heart rate of 379±6 beats/min (n=5). The blood pressure was lower, but the heart rate was not significantly different from that in control rats. Bolus injections of theophylline induced increases in mean arterial blood pressure and heart rate; however, the increasing responses were smaller than those of rats not treated with reserpine. Although the peripheral blood flow, like that in control rats, was transiently increased by a bolus injection of acetylcholine, it was never increased by the injection of theophylline. These mean arterial blood pressure, heart rate, and peripheral blood flow responses to theophylline at a dose of 5 mg/kg, i.v. of rats treated with reserpine together with those of control rats are shown in Fig. 1. Figure 2 shows the responses to acetylcholine at a dose of 1 μg/kg, i.v. in the peripheral blood flow in control and reserpinized rats, which are the same as those in Fig. 1. Average baseline values of peripheral blood flow (108±8 arbitrary units in the control rat group and 103±7 arbitrary units in the reserpinized rat group) and average maximal values after the injection of acetylcholine (191±21 arbitrary units in the control rat group and 185±13 arbitrary units in the reserpinized rat group) showed no significant difference between the rat groups.

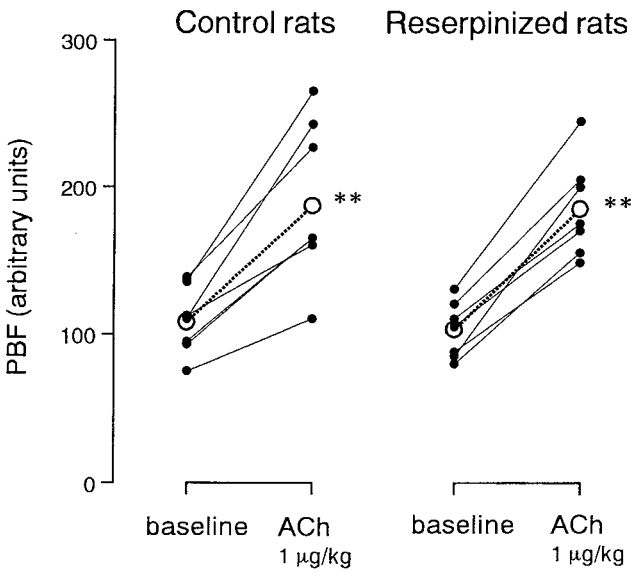

Fig. 2. Responses to acetylcholine in peripheral blood flow (PBF) in control and reserpinized rats. Rats were the same as in Fig. 1. Responses were examined before injections of theophylline. Average values of arbitrary units (○) of baseline and maximal values after acetylcholine are expressed as means±S.E.M. **P<0.005 versus baseline values.

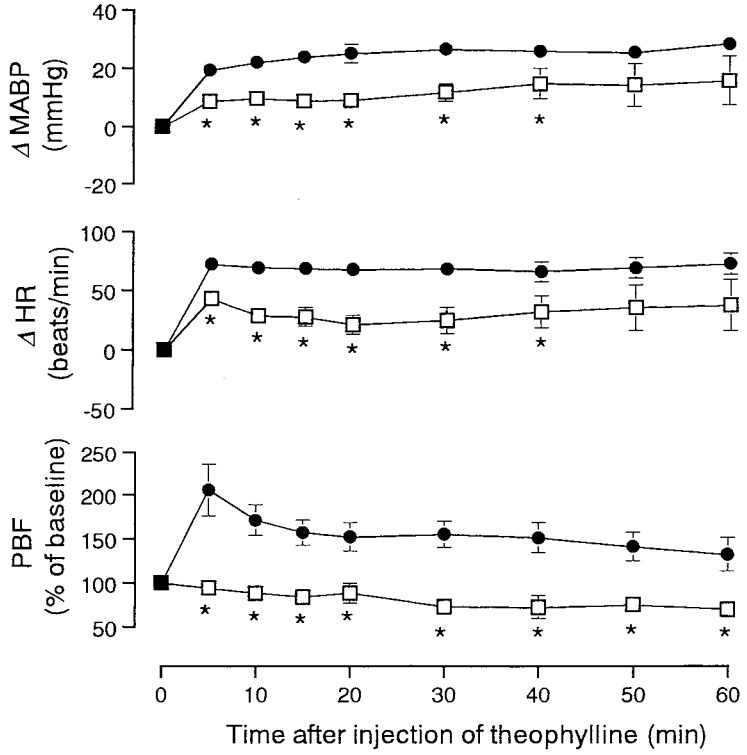

Fig. 1. Changes in mean arterial blood pressure (ΔMABP), heart rate (ΔHR) and peripheral blood flow in rat ear (PBF) following an i.v. bolus of theophylline (5 mg/kg) to control rats (n=7, ●) and to reserpine-treated rats (n=5, □). Each point represents the mean±S.E.M. Where no error bar is shown, the error is smaller than the symbol. *P<0.05 versus control rats.

Effects of propranolol and atropine in the absence and presence of theophylline

Propranolol (0.5 mg/kg and then 1.0 mg/kg) caused a clear attenuation of heart rate, although it caused only small changes in arterial blood pressure and peripheral blood flow in control rats (Fig. 3a). In theophylline-treated rats, 0.5 mg/kg propranolol caused attenuation of the increases in heart rate by theophylline and had little influence on increases in arterial blood pressure and peripheral blood flow, and 1.0 mg/kg propranolol nearly canceled the increases in arterial blood pressure and heart rate and attenuated, but not canceled, the increase in peripheral blood flow (Fig. 3b).

Atropine (1.0 mg/kg) caused an increase in heart rate without changes in arterial blood pressure and peripheral blood flow in control rats (Fig. 4a). In theophylline-treated rats, the theophylline-induced increases in arterial blood pressure, heart rate and peripheral blood flow were not influenced by atropine (Fig. 4b).

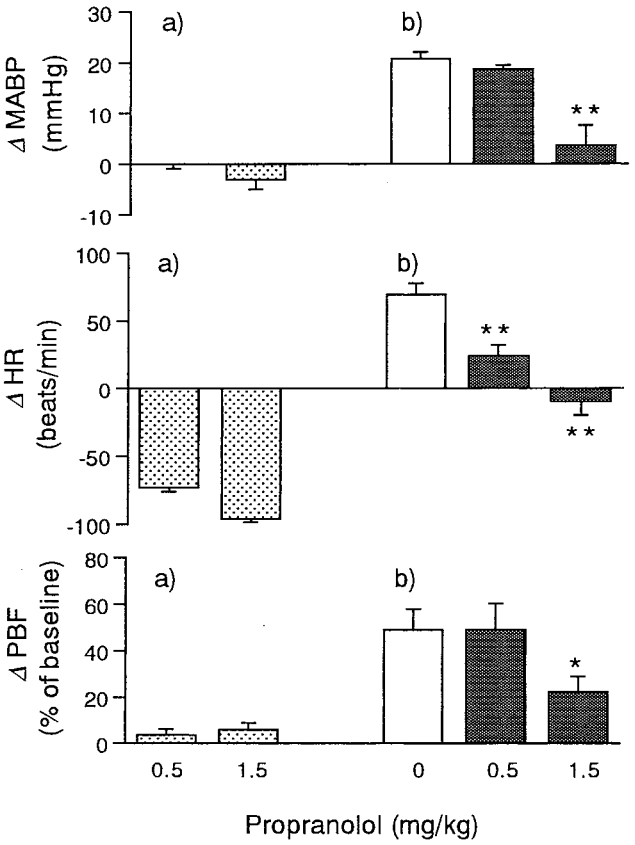

Fig. 3. Effects of propranolol on mean arterial blood pressure (ΔMABP), heart rate (ΔHR) and peripheral blood flow in ear (ΔPBF) in control rats and theophylline-treated rats. Propranolol (0.5 mg/kg) followed by propranolol (1.0 mg/kg) was injected i.v. into a) control rats and b) theophylline (5 mg/kg)-treated rats. Values are expressed as means±S.E.M. *P<0.05, **P<0.005 versus theophylline-induced values.

Effects of L-NAME, D-NAME and L-NMMA in the presence of theophyllin

A bolus injection of L-NAME at a dose of 0.3 mg/kg had little influence on blood pressure, heart rate, or peripheral blood flow of anesthetized rats. However, when the increased level in peripheral blood flow reached a plateau 15 min after an injection of theophylline, a bolus injection of L-NAME at a dose of 0.3 mg/kg reversed the increase in peripheral blood flow within 20 min and returned it to the baseline, without changes in mean arterial blood pressure or heart rate (Fig. 5). The reversal by L-NAME always had a lag time of 5–7 min from the injection. On the other hand, D-NAME at a dose of 0.5 mg/kg and L-NMMA at a dose of 1.0 mg/kg did not reverse the increase in peripheral blood flow (Figs. 5 and 6). The dose of each agent had little influence on blood pressure, heart rate or peripheral blood flow of anaesthetized rats. L-NMMA at a dose of 2.0 mg/kg, which immediately evoked an increase in arterial blood pressure with sometimes further increase in peripheral blood flow, attenuated the flow increase, but the effect of L-NMMA was not significant statistically within 20 min after the substance injection (not shown).

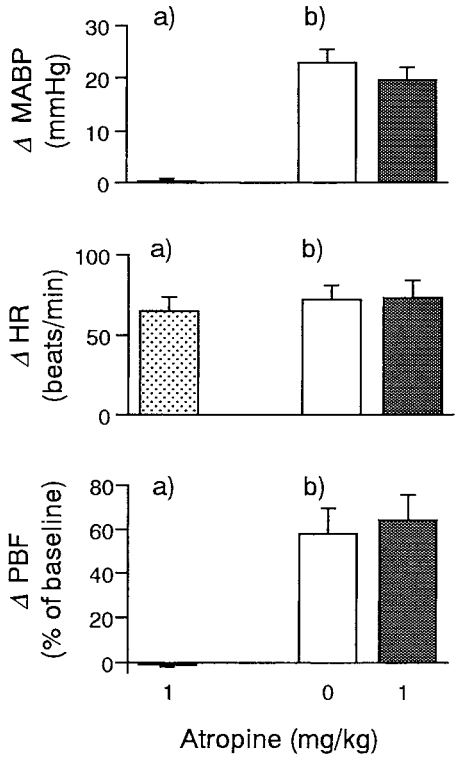

Fig. 4. Effects of atropine on mean arterial blood pressure (ΔMABP), heart rate (ΔHR) and peripheral blood flow in ear (ΔPBF) in control rats and theophylline-treated rats. Atropine (1.0 mg/kg) was injected i.v. into a) control rats and b) theophylline (5 mg/kg)-treated rats. Values are expressed as means±S.E.M.

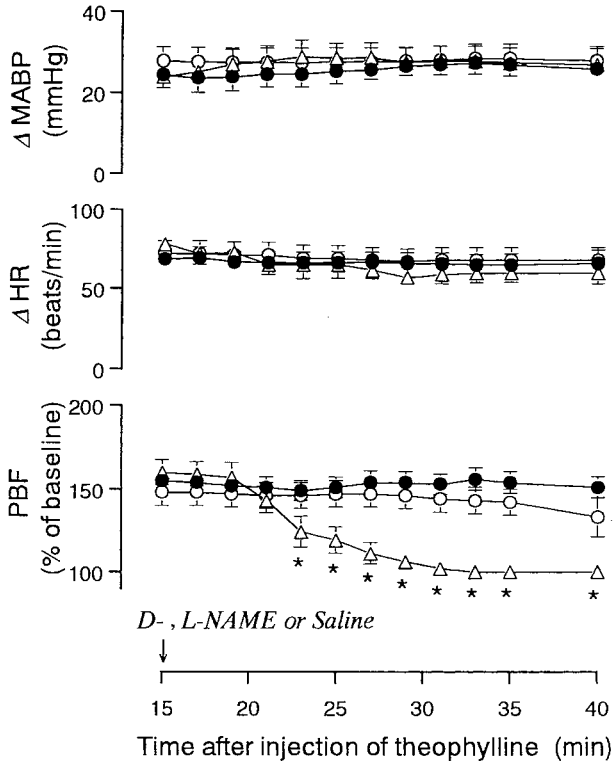

Fig. 5. Effects of a bolus of N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-D-arginine methyl ester (D-NAME) or saline on theophylline-induced changes in mean arterial blood pressure (ΔMABP), heart rate (ΔHR) and peripheral blood flow in rat ear (PBF). L-NAME (0.3 mg/kg) (n=7, △), D-NAME (0.5 mg/kg) (n=4, ○) or saline (n=7, ●) was administered 15 min after injection of theophylline (5 mg/kg). Each point represents the mean±S.E.M. *P<0.05 versus saline.

Effects of L-NAME on response of peripheral blood flow to acetylcholine

The peripheral blood flow of rat ear was increased by acetylcholine (1 μg/kg, i.v.). The flow response to acetylcholine was not canceled but significantly reduced to 90.3±3.8% of first response by treatment with L-NAME (0.3 mg/kg). Saline did not influence the flow response to acetylcholine (98.9±2.3% of first response) (Fig. 7).

Effects of atropine and propranolol, L-NAME, and L-NMMA on response of peripheral blood flow to theophylline

In the presence of atropine and propranolol, the peripheral blood flow was increased by theophylline, but the response were significantly smaller than that of the saline conrol. In the presence of L-NAME (0.3 mg/kg), the responce of peripheral blood flow to theophylline was not significant. On the contrary, a response to theophylline in the presence of L-NMMA (1.0 mg/kg) was observed as well as that in control group (Fig. 8).

DISCUSSION

In the case of rats anesthetized by α-chloralose and urethane in this study, a bolus of theophylline produced an increase in peripheral blood flow in the animal's ear as determined by laser-Doppler flowmetry, with a rise in arterial blood pressure and tachycardia in a dose-dependent manner. However, in the animals first treated with reserpine, we did not observe any increase in peripheral blood flow by theophylline, and the effects on arterial blood pressure and heart rate were significantly attenuated. On

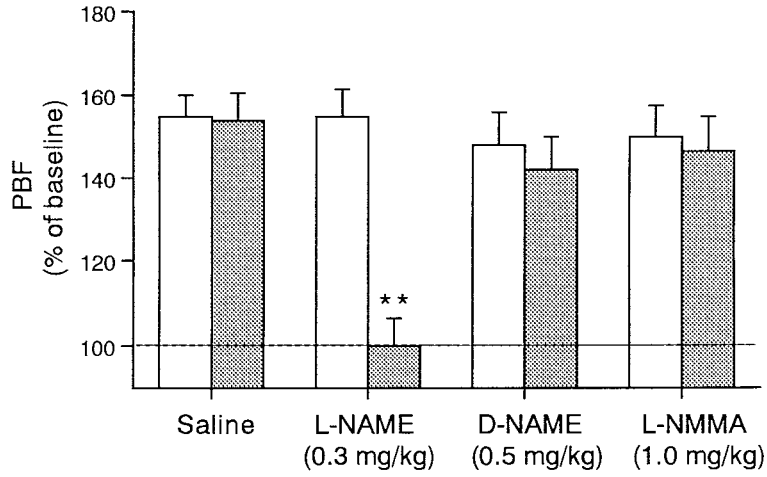

Fig. 6. Effect of L-arginine analogues on theophylline-induced changes in peripheral blood flow in rat ear (PBF). Saline, N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-D-arginine methyl ester (D-NAME) or N^G-monomethyl-L-arginine (L-NMMA) was administered 15 min after injection of theophylline (5 mg/kg). Open columns and dotted columns represent PBFs before and 20 min after saline or L-arginine analogues, respectively. Values are expressed as means±S.E.M. **P<0.005 versus theophylline-induced PBF before L-arginine analogues.

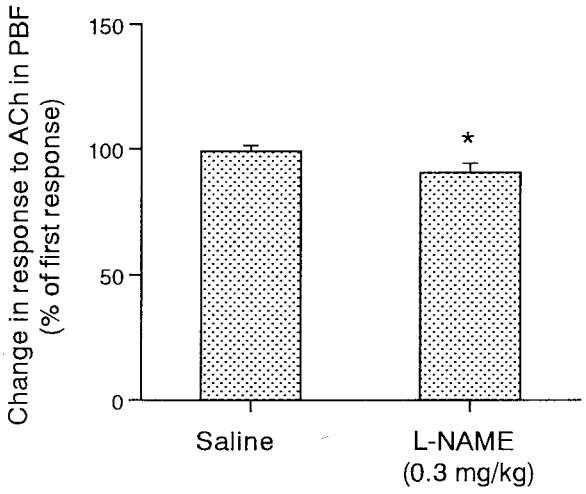

Fig. 7. Influence of N^G-nitro-L-arginine methyl ester (L-NAME) on response to acetylcholine in peripheral blood flow in rat ear (PBF). Saline or L-NAME (0.3 mg/kg) were administered 15 min before a 2nd injection of acetylcholine (ACh, 1 μg/kg). Values are expressed as means±S.E.M. of the 2nd PBF response to ACh. *P<0.05 versus saline.

the other hand, reserpine had no effect on the blood flow increase produced by acetylcholine. These results suggest that the regional and cardiac hemodynamic effects of theophylline are caused, at least in part, by the release of endogenous catecholamines, thus confirming previous reports (14, 15). Theophylline is a potent stimulant of the central nervous system and has also been reported to stimulate the release of epinephrine and norepinephrine from the sympathetic nervous system (1, 16, 17). Vestal et al. (17) found consecutive and increased catecholamine levels after aminophylline infusion in humans. Reserpine abolished the increase of peripheral blood flow, but not the arterial blood pressure and heart rate caused by theophylline. The theophylline-induced changes in arterial blood pressure and heart rate of reserpinized rats, which were distinctly smaller than those of control rats, were probably due to a direct action on the heart by theophylline through phosphodiesterase inhibition.

Norepinephrine and epinephrine have both inotropic and chronotropic effects on the heart, which, with a vasoconstrictor effect, lead to an increase in systolic blood pressure. Therefore, the change in peripheral blood flow may result from primary actions of theophylline on the heart, with modification by autonomic reflexes and changes in the concentrations of circulating catecholamines. Indeed, a β-adrenergic blocker, propranolol, attenuated the theophylline-induced increases in these parameters. However, a low dose of propranolol (0.5 mg/kg), which caused attenuations of the increases in blood pressure and heart rate, did not change the peripheral blood flow in theophylline-treated rats. Atropine (1.0 mg/kg), inducing an increase in heart rate, also did not cause a change in the peripheral blood flow. Moreover, theophylline-induced flow increases were observed in the presence of atropine (1.0 mg/kg) and propranolol (1.0 mg/kg), but bolus doses of both agents given together abolished the reflex heart rate. These find-

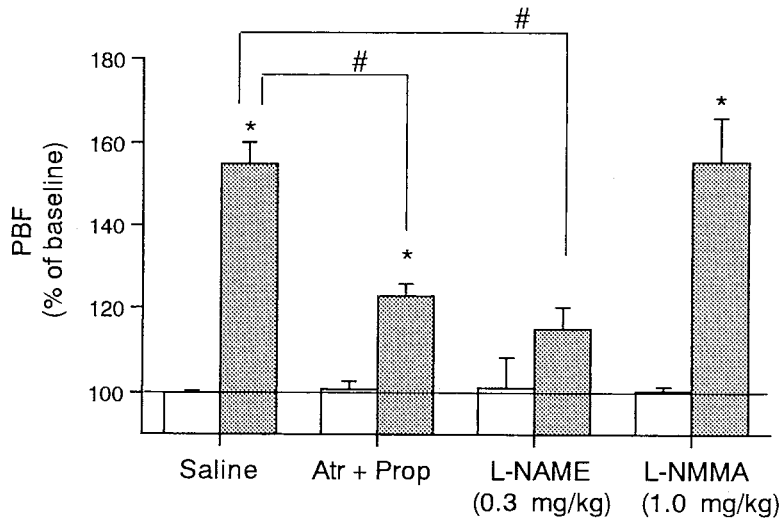

Fig. 8. Effects of preliminary treatment with atropine and propranolol, N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA) on theophylline-induced increase in peripheral blood flow in rat ear (PBF). Saline, atropine (Atr, 1.0 mg/kg) and propranolol (Prop, 1.0 mg/kg), L-NAME, or L-NMMA was administered 15 min before injection of theophylline (5 mg/kg). Open columns and dotted columns represent PBFs before and 15 min after thophylline, respectively. Values are expressed as means±S.E.M. *P<0.05 versus before theophylline. #P<0.005 versus saline control.

ings suggest that an independent effect from the response to changes in blood pressure and heart rate is involved in the change in peripheral blood flow by theophylline, although only the frequent and initial large increase in peripheral blood flow after injection of theophylline is possibly a result of the primary actions on the heart. In this study, we deal with the persistent increase in peripheral blood flow induced by theophylline, which is dissociated from the effects on blood pressure and heart rate.

Sympathetic stimulation causes changes in vascular resistance that are mediated by epinephrine, norepinephrine and α_1-, α_2- and β-adrenoceptors. Although the response to catecholamines has significant variability between the specific vascular beds, and involves differences in either the α-adrenoceptor-mediated vasoconstrictor component, the β-adrenoceptor mediated vasodilator component, and/or nitric oxide (NO) production. It has been proposed that the vasoconstrictor response to norepinephrine was considerably less in small compared to large pulmonary arteries, and this diversity in response was partially due to the β-adrenoceptor-mediated vasodilator component (18, 19). In our experiments, the effects of theophylline on arterial blood pressure, heart rate, and peripheral blood flow were constantly produced 15 min after a bolus of theophylline (5 mg/kg) and were maintained for at least 60 min when no further drug was administered. Subsequent administration of propranolol attenuated, but did not cancel, the theophylline-induced flow increase, although it nearly canceled the increases in arterial blood pressure and heart rate. The result indicates that theophylline-induced increase in peripheral blood flow involves a β-adrenoceptor-nonmediated component(s) in addition to the β-adrenoceptor-mediated one. In addition, subsequent administration of a NO-synthase inhibitor, L-NAME (0.3 mg/kg), the dose of which alone had little effect on the baselines of mean arterial blood pressure, heart rate, and peripheral blood flow, completely reversed the theophylline-induced flow increase, without large changes in mean arterial blood pressure and heart rate. These findings suggest that theophylline-induced increase in peripheral blood flow is predominantly caused by L-NAME-sensitive mechanisms, in which the β-adrenoceptor-mediated component is involved partially.

NO is a vasoactive substance produced by a reaction catalyzed by NO synthase. One form of NO synthase present in vascular endothelial cells generates NO that produces relaxation of smooth muscle tone and vasodilation (9). It has been proposed that both α- and β-adreoceptor activation can lead to release of endothelial NO (20–23). Although, in this study, L-NAME (0.3 mg/kg) significantly reduced the flow response to acetylcholine, the effect was very small. Therefore, the effect of L-NAME on endothelium, which blocks the L-arginine-NO pathway, is unlikely to be a predominant factor in the reversal in peripheral blood flow by L-NAME. NO other than endothelium-derived NO can mediate the flow increase by theophylline.

The other NO-synthase inhibitor, L-NMMA, did not have a reversing effect on the peripheral blood flow response to theophylline, unlike the effect of L-NAME. Our most interesting finding is a difference between the effects of the two NO-synthase inhibitors. It has been proposed that L-NAME interacts in a complex fashion with various systems including the L-arginine-NO system in cardiovascular regulation (24) and the cholinergic system (25). However, although administrations of L-NAME to experimental animals causes widespread vasoconstriction, increased peripheral vascular resistance, and arterial hypertension (9), and a fall in cardiac output in rats (24), it is unlikely that the reversal in peripheral blood flow could be explained by autonomic reflexes and a reduction in cardiac function with a resultant increase in sympathetic tone, since the reversal by L-NAME was always accompanied by a lag time. Although L-NAME, but not L-NMMA, also antagonizes the muscarinic receptor (25), it is unlikely that the reversal in peripheral blood flow could be explained by inhibition of the cholinergic system, since the theophylline-induced flow increase was not influenced by atropine.

Recently NO has emerged as the major nonadrenergic, noncholinergic (NANC) transmitter in the peripheral nervous system (26), and the interactions between cholinergic and adrenergic or NANC nerves, and between adrenergic and NANC nerves has been speculated (27). Marin et al. (28) have indicated that L-NAME, but not L-NMMA, blocks NANC relaxation in the bovine retractor penis and speculated that the diversity in responses to L-NAME and L-NMMA is due to a complex interaction between L-NAME, L-NMMA and the endogenous precursor, L-arginine, at the level of the neuronal NO-synthase. Moreover, Zhang et al. (29) have indicated that nicotine-induced NO-mediated relaxation in porcine cerebral arteries was abolished by denervation by 6-hydroxydopamine, and suggested that nicotine act on the presynaptic adrenergic nerve terminals to release norepinephrine or a related substance, which then stimulates release of NO from the neighboring NANC nerves. In this study, the theophylline-induced flow increase in rat ear, which was L-NAME-sensitive, was abolished by adrenergic blockade by reserpine. Thus, it is feasible that theophylline stimulates not only adrenergic nerves but also NANC nerves in rat ear, causing releases of catecholamines and NO, which induce vasodilation via increase in intracellular cyclic AMP and cyclic GMP. Theophylline appears to act on NANC nerves through stimulation of

the adrenergic nerve.

Neuronal control of vascular tone is also provided by reflex responses of the central nervous system, in which the L-arginine-NO pathway mediates neurotransmission or neuromodulation (30). The inhibition of the NO pathway in brain by L-NAME, a lipophilic ester, which is more likely to enter the central nervous system than L-NMMA, may attenuate the stimulation of the central nervous system by theophylline and cause a resultant reversal in theophylline-induced flow increase. Reserpine may also block the neuronal response of vascular tone to theophylline by its effect of reducing catecholamines in the brain.

Whatever the L-NAME-sensitive mechanisms involved, our results indicate conclusively that NO-dependent mechanisms contribute to the peripheral vasodilation induced by theophylline. Accumulations of cyclic AMP and cyclic GMP as a result of inhibiting phosphodiesterase in vascular smooth muscle by theophylline is probably small, since any affinities of theophylline for cyclic AMP- and cyclic GMP-phosphodiesterase isoenzymes are very small (31). However, theophylline-induced accumulations of cyclic AMP and cyclic GMP in vascular smooth muscle by a β-adrenoceptor mediated mechanism that is endothelium-independent and by a NO-dependent mechanism via adrenoceptor stimulation by catecholamines, which involves an endothelium-dependent mechanism might be potentiated by the inhibited phosphodiesterases, then inducing vasodilation.

In conclusion, our results suggest that theophylline-induced increase in peripheral blood flow in rat ear is not due to primary actions of theophylline on the heart with modification by autonomic reflexes and is mediated by both adrenergic and nitrergic pathways.

Acknowledgment

This study was partly supported by the special research fund of Hokuriku University.

REFERENCES

1 Rall TW: Drugs used in the treatment of asthma. *In* The Pharmacological Basis of Therapeutics, Edited by Goodman Gilman A, Rall TW, Nies AS and Taylor P, pp 618–637, Pergamon Press, New York (1990)
2 Fredholm BB and Persson CGA: Xanthine derivatives as adenosine receptor antagonists. Eur J Pharmacol **91**, 673–676 (1982)
3 Ogilvie RI, Fernandez PG and Winsberg F: Cardiovascular response to increasing theophylline concentrations. Eur J Clin Pharmacol **12**, 409–414 (1977)
4 Fredholm BB: Cardiovascular and renal actions of methylxanthines. Prog Clin Biol Res **158**, 303–330 (1984)
5 Furchgott RF and Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature **288**, 373–376 (1980)
6 Berkenboom G, Fang ZY, Unger P, Goldman M and Fontaine J: Endothelium-dependent effects of pentoxifylline in rat aorta. Eur J Pharmacol **193**, 81–86 (1991)
7 Hatano Y, Mizumoto K, Yoshiyama T, Yamamoto M and Iranami H: Endothelium-dependent and -independent vasodilation of isolated rat aorta induced by caffeine. Am J Physiol **269**, H1679–H1684 (1995)
8 Marukawa S, Hatake K, Wakabayashi I and Hishida S: Vasorelaxant effects of oxpentifylline and theophylline on rat isolated aorta. J Pharm Pharmacol **46**, 342–345 (1993)
9 Moncada S, Palmer RMJ and Higgs EA: Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev **43**, 109–142 (1991)
10 Fukuto JM, Chiang K, Hszieh R, Wong P and Chaudhuri G: The pharmacological activity of nitric oxide and/or endothelium-derived relaxing factor. J Pharmacol Exp Ther **263**, 546–551 (1992)
11 Liu SF, Crawley DE, Evans TW and Barnets PJ: Endogenous nitric oxide modulates adrenergic neural vasoconstriction in guinea-pig pulmonary artery. Br J Pharmacol **104**, 565–569 (1991)
12 Fukuda O, Endo S, Kuwayama N, Harada J and Takaku A: The characterstics of laser-Doppler flowmetry for the measurement of regional cerebral blood flow. Neurosurgery **36**, 358–364 (1995)
13 Obeid AN, Dougherty G and Pettinger S: In vivo comparison of a twin wavelength laser Doppler flowmeter using He-Ne and laser diode sources. J Med Engineer Technol **14**, 102–110 (1990)
14 Rutherford JD, Vatner SF and Braunwald E: Effects and mechanism of action of aminophylline on cardiac function and regional blood flow distribution in conscious dogs. Circulation **63**, 378–387 (1981)
15 Westfall DP and Fleming WW: Sensitivity changes in the dog heart to norepinephrine, calcium and aminophylline resulting from pretreatment with reserpine. J Pharmacol Exp Ther **159**, 98–106 (1968)
16 Mackay AD, Baldwin CJ and Tattersfield AE: Action of intravenously administered aminophylline on normal airways. Am Rev Respir Dis **127**, 609–613 (1983)
17 Vestal RE, Eiriksson CE Jr, Musser B, Ozaki LK and Halter JB: Effect of intravenous aminophylline on plasma levels of catecholamines and related cardiovascular and metabolic responses in man. Circulation **67**, 162–171 (1983)
18 Leach RM, Twort CH, Cameron IR and Ward JPT: A comparison of the pharmacological and mechanical properties in vitro of large and small pulmonary arteries of the rat. Clin Sci **82**, 55–62 (1992)
19 Priest RM, Hucks D and Ward JPT: Noradrenaline, β-adrenoceptor mediated vasorelaxation and nitric oxide in large and small pulmonary arteries of the rat. Br J Pharmacol **122**, 1375–1384 (1997)
20 Graves J and Poston L: β-Adrenoceptor agonist mediated relaxation of rat isolated resistance arteries: a role for the endothelium and nitric oxide. Br J Pharmacol **108**, 631–637 (1993)
21 Gray DW and Marshall I: Novel signal transduction pathway mediating endothelium-dependent β-adrenoceptor vasorelaxation in rat thoracic aorta. Br J Pharmacol **107**, 684–690 (1992)

22 Kaneko K, Sunano S: Involvement of α-adrenoceptors in the endothelium-dependent depression of noradrenaline-induced contraction in rat aorta. Eur J Pharmacol **240**, 195–200 (1993)

23 Ohgushi M, Yasue H, Kugiyama K, Murohara T and Sakaino N: Contraction and endothelium dependent relaxation via alpha adrenoceptors are variable in various pig arteries. Cardiovasc Res **27**, 779–784 (1993)

24 Gardiner SM, Compton AM, Kemp PA and Bennett T: Regional and cardiac haemodynamic effects of N^G-nitro-L-arginine methyl ester in conscious, Long Evans rats. Br J Pharmacol **101**, 625–631 (1990)

25 Buxton IL, Cheek DJ, Eckman D, Westfall DP, Sanders M and Keef KD: N^G-Nitro-L-arginine methyl ester and other alkyl esters of arginine are muscarinic receptor antagonists. Circ Res **72**, 387–395 (1993)

26 Rand MJ and Li CG: Nitric oxide as a neurotransmitter in peripheral nerves: nature of transmitter and mechanism of transmission. Annu Rev Physiol **57**, 659–682 (1995)

27 Toda N, Yoshida K and Okamura T: Involvement of nitroxidergic and noradrenergic nerves in the relaxation of dog and monkey temporal veins. J Cardiovasc Pharmacol **25**, 741–747 (1995)

28 Martin W, Gillespie JS and Gibson IF: Actions and interactions of N^G-substituted analogues of L-arginine on NANC neurotransmission in the bovine retractor penis and rat anococcygeus muscles. Br J Pharmacol **108**, 242–247 (1993)

29 Zhang W, Edvinsson L and Lee TJF: Mechanism of nicotine-induced relaxation in the porcine basilar artery. J Pharmacol Exp Ther **284**, 790–797 (1998)

30 Garthwaite J, Garthwaite G, Palmer RMJ and Moncada S: NMDA receptor activation induces nitric oxide synthesis from arginine in rat brain slices. Eur J Pharmacol **172**, 413–416 (1989)

31 Cortijo J, Bou J, Beleta J, Cardelus I, Llenas J, Morcillo E and Gristwood RW: Investigation into the role of phosphodiesterase IV in bronchorelaxation, including studies with human bronchus. Br J Pharmacol **108**, 562–568 (1993)