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Effect of Theophylline on Hypoxic Pulmonary Vasoconstriction in Awake Rats

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ARISAKA, Y., SATO, S., KATO, S., YUKI, H., TAKAHASHI, H. and TOMOIKE, H. *Effect of Theophylline on Hypoxic Pulmonary Vasoconstriction in Awake Rats.* Tohoku J. Exp. Med., 1997, **182** (3), 231-239 —— Whether or not theophylline inhibits hypoxic pulmonary vasoconstriction in vivo still remains uncertain. We therefore studied the effect of theophylline on hypoxic pulmonary vasoconstriction in awake rats. Two days before hemodynamic measurement, indwelling catheters were placed. Animals were divided into three groups; Group-H (20 mg/kg of theophylline), Group-L (8 mg/kg of theophylline), Group-S (saline). At the day of hemodynamic measurement, animals breathed 21% and 10% O_2 gas. [{Pulmonary vascular resistance (PVR) during 10% O_2-PVR during 21% O_2}/PVR during 21% O_2] $\times$ 100 was termed as hypoxic pulmonary vasoconstriction (HPV). The first HPV measurement was followed without drug administraion and then the second HPV measurement was performed with theophylline or saline infusion. Post-theophylline HPV was divided by pre-theophylline HPV to normalize individual variation. Ratio of post-theophylline HPV to pre-theophylline HPV was 0.49 ± 0.10, 0.77 ± 0.23, 1.06 ± 0.33 in Group -H, -L, -S, respectively (means $\pm$ S.E.M). Ratio of post-theophylline HPV to pre-theophylline HPV was significantly less in Group-H than in Gruop-S. This result suggests that theophylline used in the present study (18.6-26.9 μg/ml) attenuates hypoxic pulmonary vasoconstriction in the unanesthetized rat. ———— hypoxic pulmonary vasoconstriction; theophylline; unanesthetized rats; pentobarbital anesthesia

Theophylline is a popular remedy as a bronchodilator for patients not only with chronic obstructive pulmonary disease (COPD) but also with bronchial asthma. Whether theophylline inhibits hypoxic pulmonary vasoconstriction remains uncertain. In various experimental animal preparations, theophylline has been shown to inhibit (Hales and Kazemi 1974; Hakim and Petrella 1988) or to have no effect on (Benumof and Trousdale 1982) the hypoxic pulmonary pressor respose. In addition, recent study has indicated that the high dose of theophyl-

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line is needed to inhibit hypoxic pulmonary pressor response (Lejeune et al. 1989). This inconsistency may depend on the level of anesthesia, the type of anesthesia, the dose of theophylline (Lejeune et al. 1989), the species (Reeves and Herget 1984), or experimental design, regional or global hypoxia (Marshall and Marshall 1980).

In vivo, hypoxic pulmonary vasoconstriction has been studied mostly in pentobarbital anesthetized animals. Pentobarbital attenuates hypoxia-induced pulmonary vascular tone (Wetzel and Martin 1989). Since previous studies did not mention the level of pentobarbital in plasma (Hales and Kazemi 1974; Benumof and Trousdale 1982; Hakim and Petrella 1988; Lejeune et al. 1989), it is unclear whether the effects of theophylline were tested at an identical pentobarbital concentration. To eliminate the influence of pentobarbital and to simulate the clinical situation, it is desirable to examine the effect of theophylline on hypoxic pulmonary vasoconstriction in the unanesthetized state. In the present study, an awake rat model was used to assess alteration of pulmonary vascular tone by inspired hypoxia before and after theophylline.

Materials and Methods

Animal preparation

Male Wistar rats of 9–11 weeks were anesthetized with an intraperitoneal bolus injection of pentobarbital (5 mg/100 g body weight). After a small incision was made in the neck, a Silastic catheter (0.3 mm inner diameter, 0.6 mm outer diameter, Dow Corning Co., Midland, MI, USA) filled with heparinized saline solution was advanced from the right external jugular vein to the pulmonary artery, while monitoring the pressure (Rabinovitch et al. 1979). The left external jugular vein was isolated, and a catheter (PE-20; Becton, Dickinson and Co., Parsippany, NJ, USA) was inserted into the left jugular vein for drug administration. After a midline incision had been made in the abdomen, the abdominal aorta was exposed just above the iliac bifurcation. A polyethylene catheter (PE-10 fused to PE-50; Becton, Dickinson and Co.) filled with heparinized saline solution was inserted into the aorta (Weeks and Jones 1960). After all three catheters had been flushed and filled with heparinized saline (1000 U/ml), the end of the catheters were closed with a blunted wire plug. Then, the three catheters were tunneled subcutaneously to the base of skull and were brought to the exterior.

Experimental protocol

Two days after the catheter insertion, a hemodynamic study was performed in the unanesthetized state while the rat was placed in a clear Lucite chamber of 1-liter capacity as described previously (Fried et al. 1983).

To obtain baseline hemodynamic data in 21% O_2 before theophylline administration, the chamber was filled with room air at 5 liter/min for the initial 2

minutes and then at 2 liter/min. After 20 minutes of equilibration, the chamber was switched to a closed system and was connected to a Krogh respirometer to measure O_2 consumption ($\dot{V}O_2$). During this procedure, pulmonary artery pressure (Ppa) and systemic artery pressure (Psa) were continuously monitored and $\dot{V}O_2$, hemoglobin content (Hb), and mixed venous and aortic O_2 saturation ($S\bar{v}O_2$, SaO_2) were measured (first measurement in 21% O_2). Blood gas levels were also analyzed.

After completion of the first hemodynamic measurement, 10% O_2 gas was blown into the chamber and the hemodynamic variables along with $\dot{V}O_2$, SaO_2 and $S\bar{v}O_2$ were measured after 20 to 25 minutes equilibration in 10% O_2 (first measurement in 10% O_2). The first measurement in 10% O_2 was followed by breathing with room air. Five minutes later, theophylline (0.1 ml/200 g) at a dose of 20 mg/kg (Group-H) or 8 mg/kg (Group-L) or saline solution (Group-S) was infused into the left jugular vein catheter for 10 minutes using a constant-rate infusion pump (Harvard Model 22; Harvard Apparatus, South Natick, MA, USA). Hemodynamic variables were determined 10 minutes after the end of theophylline infusion (second measurement in 21% O_2).

Finally, the effect of theophylline on hemodynamic variables in 10% O_2 gas were studied (second measurement in 10% O_2). At the end of these four measurements, 0.4 ml of arterial blood was collected and the plasma concentration of theophylline was measured by a fluorescence polarization immunoassay technique (Jolley et al. 1981).

Hemodynamic measurements and calculation of resistance

Pulmonary artery and systemic artery pressures were measured using strain gauge transducers (TP-101T; Nihon Kohden, Tokyo) and were processed with carrier amplifiers. The zero level of the pressure transducers was set at the mid-thoracic point in the resting prone position. Thirty second averages of filtered recording of Ppa and Psa were determined and respective vascular resistances were calculated. Blood samples (0.2 ml each) were drawn into heparinized capillaries from the pulmonary artery and the aorta for measurements of oxygen saturation and hemoglobin concentration. They were analyzed by an IL-282 CO-Oximeter (Instrumentation Laboratories, Lexington, MA, USA). Blood gases (PaO_2, $PaCO_2$, and pH) were measured by an IL-813 blood gas analyzer (Instrumentation Laboratories). In the present experiments, the total blood volume required for four consecutive measurements was about 2 ml, and in each sampling an equivalent amount of heparinized saline solution was intravenously administered.

To measure oxygen consumption, CO_2 and H_2O in the expired gas inside the chamber were absorbed through CO_2 absorber and desiccant silica gel in the chamber, respectively. Consumed O_2 was replaced by 100% O_2 from the Krogh respirometer. The amount of consumed oxygen was measured by the movement

of the respirometer attached to a linear displacement transducer and was monitored on the recorder. The system was run, refilling the respirometer with 100% O_2 until a stable linear utilization of O_2 and thermal equilibrium were achieved (about 7 minutes). Oxygen consumption was calculated over the course of 5 minutes, during which time blood was sampled, correcting the volume to standard temperature, pressure and dry (STPD).

Cardiac output (C.O.) was calculated using the following formula;

$$C.O. = \dot{V}O_2 / \{1.39 \times Hb \times (SaO_2 - S\bar{v}O_2) \times 10^{-2}\}$$

The unit of Hb, SaO_2 and $S\bar{v}O_2$, or C.O. is g/100 ml, %, or ml/min, respectively.

Pulmonary vascular resistance (PVR) and systemic vascular resistance (SVR) were defined as follows;

$$PVR = (Ppa - 4.7)/C.O.$$
$$SVR = Psa/C.O.$$

For the level of left atrial pressure, 4.7 cmH_2O was used (Meyrick et al. 1980), and 0 cmH_2O was assumed for the right atrial pressure.

The concept of hypoxic pulmonary vasoconstriction is numerically best expressed as a percent increase above baseline. Accordingly, [(PVR during 10 % O_2-PVR during 21% O_2)/PVR during 21% O_2] $\times$ 100 was calculated and termed hypoxic pulmonary vasoconstriction (HPV). Since HPV showed big variations among rats in the preliminary experiment, post-theophylline HPV was divided by pre-theophylline HPV to normalize rat-to-rat variation. This value was defined as the ratio of post-theophylline HPV to pre-theophylline HPV.

Statistical analysis

Data are expressed as mean $\pm$ S.E.M. To compare the hemodynamic changes among three groups, an ANOVA test was applied. Student's *t*-test for paired values was used to compare hemodynamic data before and after drug administration in each group. A $p < 0.05$ was considered significant.

Results

Body weight, hemoglobin concentration, arterial blood pH, PaO_2 and $PaCO_2$ at the first hemodynamic measurement were generally within the physiological ranges. These parameters were not significantly different among three groups.

Total plasma theophylline concentration at the end of the experiment was 22.1 $\pm$ 1.2 μg/ml, (range 18.6-26.9 μg/ml) in Group-H and 8.1 $\pm$ 0.5 μg/ml (range 6.5-9.4 μg/ml) in Group-L. Free theophylline concentration was 10.4 $\pm$ 1.1 μg/ml (range 8.8-17.8 μg/ml) in Group-H and 4.4 $\pm$ 0.4 μg/ml (range 4.0-6.4 μg/ml) in Group-L. Neither convulsions nor emesis was observed in conscious rats after intravenous infusion of theophylline.

Immediately after the room air in the chamber was replaced with hypoxic gas, the rat usually showed unstable behavior and occasional deep respirations

TABLE 1. *Hemodynamic changes by hypoxia and theophylline*

	Inspired O_2 (%)	Ppa (cmH_2O)	PVR ($cmH_2O \cdot min \cdot kg/ml$)	Psa (cmH_2O)	SVR ($cmH_2O \cdot min \cdot kg/ml$)	C.I. (ml/min/kg)	HR (beat/min)
Before theophylline or saline infusion							
Group-H ($n=8$)	21	22.8±1.0	0.054±0.006	160±6	0.48±0.05	351±26	458±15
	10	28.3±1.0**	0.067±0.005**	146±7**	0.42±0.03*	358±14	498±15*
Group-L ($n=8$)	21	24.1±0.5	0.054±0.002	156±6	0.43±0.03	357±16	428±13
	10	27.8±1.2**	0.065±0.003**	142±8**	0.40±0.02*	357±15	475±17**
Group-S ($n=7$)	21	22.3±1.6	0.055±0.006	157±7	0.50±0.04	319±22	456±5
	10	27.8±1.9**	0.069±0.006**	148±7*	0.45±0.02*	334±16	499±16*
After theophylline or saline infusion							
Group-H ($n=8$)	21	24.5±1.6	0.061±0.006	154±6	0.47±0.04	338±19	498±13‡
	10	27.2±1.6*	0.067±0.006**	137±5**,†	0.41±0.04	346±24	555±34†
Group-L ($n=8$)	21	25.6±1.6	0.063±0.005	154±5	0.48±0.03	337±17	484±16‡
	10	27.7±1.3*	0.069±0.004**	137±7*	0.42±0.03**	331±14	527±11*,†
Group-S ($n=7$)	21	26.0±2.8	0.061±0.008	158±8	0.46±0.03	348±25	467±19
	10	27.9±2.0*	0.072±0.007**	137±9**	0.44±0.04	321±23	493±15*

Ppa, pulmonary arterial pressure; PVR, pulmonary vascular resistance; Psa, systemic arterial pressure; SVR, systemic vascular resistance; C.I., cardiac index; *$p<0.05$ vs. preceding 21% O_2; **$p<0.01$ vs. preceding 21% O_2; †$p<0.05$ vs. 10% O_2 before theophylline infusion; ‡$p<0.05$ vs. 21% O_2 before theophylline infusion.

were noted along with tachypnea. Pulmonary arterial pressure gradually reached a plateau level within several minutes. Aortic pressure decreased. With or without drug administration, all rats showed an increase in PVR during hypoxia and the increase was statistically significant in all groups (Table 1) ($p<0.01$).

Ppa, PVR and other variables before and during hypoxia, in the absence or presence of the drug, are summarized in Table 1. Ppa was significantly increased by hypoxia in all groups, either before ($p<0.01$) or after ($p<0.05$) theophylline infusion.

Psa before theophylline infusion decreased by 20.8 ± 3.9, 16.5 ± 5.0 and 18.1 ± 4.0 cmH_2O after hypoxia in Group-S, -L and -H, respectively. Aortic depressant effect of hypoxia was not statistically different among the three groups.

Heart rate increased during hypoxia and after theophylline administration in each group (Table 1). Cardiac index (C.I.), however, was not significantly changed either by hypoxic exposure or by theophylline administration.

Pre-theophylline and Post-theophylline HPV in each group is shown in Table 2. HPV in Group-H significantly decreased by theophylline infusion.

The ratio of post-theophylline HPV to pre-theoppyllie HPV was dose-dependently decreased. This values was 0.49 ± 0.10, 0.77 ± 0.23, 1.06 ± 0.33 in Group-H, -L, -S, respectively (means$\pm$S.E.M.) (Table 2).

Discussion

In the literature, whether theophylline inhibits hypoxic pulmonary vasoconstriction in vivo still remains controversial. Aminophylline has been shown to decrease blood diversion from one lung made hypoxic in intact anesthetized dogs (Hales and Kazemi 1974). Hypoxia-induced blood flow diversion from an in vivo canine left lower lobe preparation was preserved after administration of aminophylline (Benumof and Trousdale 1982). Only the high dose of theophylline reduced hypoxic pulmonary pressor response in intact anesthetized dogs (Lejeune et al. 1989). We suggest that conflicting results may be explained by the fact that previous research in vivo has always involved the use of anaesthetics. In addition, these discrepancies may be affected at least in part by differences in theo-

Table 2. *Change of HPV by theophylline in three groups*

	Pre-theophylline HPV (%)	Post-theophylline HPV (%)	Post-theophylline HPV / Pre-theophylline HPV
Group-H ($n=8$)	27.4 ± 6.5	13.7 ± 4.9*	0.49 ± 0.10†
Group-L ($n=8$)	19.1 ± 4.0	12.6 ± 3.7	0.77 ± 0.23
Group-S ($n=7$)	25.6 ± 6.5	21.2 ± 7.3	1.06 ± 0.33

Data are presented as mean$\pm$S.E.M. Student's t-test for paired values was performed to compare the values in each group and ANOVA was used for unpaired values.

*$p<0.05$ when compared with pre-theophylline HPV; †$p<0.05$ when compared with Group-S.

phylline doses and size of hypoxia. The aim of the present study was to elucidate the effect of two doses of theophylline on hypoxic pulmonary vasoconstriction in awake rats, by global hypoxia. We present the data that theophylline, in a dose-dependent fashion, blunts hypoxic pulmonary vasoconstriction induced by global hypoxia in awake rats.

Pulmonary artery and aortic pressures and cardiac output were measured in unanesthetized rats. Since the Fick equation requires a stable state of hemodynamics, $\dot{V}O_2$ in the unanesthetized rat was determined when the body movements were minimum. Ppa and Psa were also averaged for every 30 seconds during $\dot{V}O_2$ measurements for 5 minutes.

Since the pulmonary wedge pressure was not measurable in our rats, we used the value of 4.7 cmH_2O for the left atrial pressure (Meyrick et al. 1980). Whether the left atrial pressure changes during hypoxia or theophylline administration remained unexamined in rats. However, in dogs (Lejeune et al.1989), left atrial pressure was unchanged by hypoxia or theophylline. Therefore, the pre-set of left atrial pressure to the level of 4.7 cmH_2O was not unreasonable.

Hypoxic pulmonary vasoconstriction is modified not only by PaO_2 but also by Hb (McGrath and Weil 1978), $PaCO_2$ (Noble et al. 1981) and pH (Lloyd 1966). However, they were not significantly different among the three groups at the first hemodynamic measurement. Thus, the change of HPV in the present study mainly reflects the effect of theophylline per se on hypoxic pulmonary vasoconstriction response.

The therapeutic concentration of theophylline in patients with bronchial asthma has been reported to be about 4–20 μg/ml (Jenne et al. 1972; Hendels and Weinberger 1980). In the present study, the plasma concentration of theophylline was about 20 μg/ml in Group-H and 8 μg/ml in Group-L which correspond to the therapeutic upper concentrations in human.

The elevated level of pulmonary intravascular pressure and/or increased distension of the vascular wall have been reported to blunt the hypoxic pulmonary vasoconstriction response (Benumof and Wahrenbrock 1975; Tucker et al. 1978). The higher level of Ppa and PVR before hypoxia may blunt HPV. Since, there was no significant difference in Ppa and PVR before hypoxia among three groups in the present study, it is unlikely that these factors affected HPV. The Ppa and PVR before hypoxia tended to be slightly raised by theophylline (Table 1). However, the level of Ppa and PVR before hypoxia did not differ statistically between pre-theophylline and post-theophylline in each group. Accordingly, the present protocol would have been sufficient to detect the direct modulating the effect of theophylline per se on hypoxic pulmonary vasoconstriction response.

In the present study, although HR was significantly elevated during hypoxia and theophylline administration, it had little effect on C.O. Perhaps, increasing HR caused shortening of ventricular filling time, reduction of the ventricular

end-diastolic volume and reduced extension of cardiac muscle fibers, resulting in decreased stroke volume.

Theophylline reduced dose-relatedly the ratio of post-theophylline HPV to pre-theophylline HPV, although in terms of pulmonary artery pressure, the absolute level of this modulation was not big. The finding that theophylline induced a dose-dependent attenuation of hypoxic pulmonary vasoconstriction without significant changes in C.O., systemic vascular resistance or PaO_2, suggests that it may be suitable for the treatment of patients with COPD complicated with pulmonary hypertension. However, in man the net benefit is obscured because ventilation-perfusion inequalities reduce arterial blood oxygen tension (Jezek et al. 1970).

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