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Effects of pirarubicin, an antitumor antibiotic, on the cardiovascular system

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Summary. In the present study we examined the effects of pirarubicin [(2″*R*)-4′-*O*-tetrahydropyranyladriamycin, THP] on a cardiovascular system. An injection of THP (0.39–3.13 mg/kg, i.v.) reduced the mean blood pressure and caused an increase in the respiratory air rate in anesthetized rats. At 1.5×10^{-6}–1.5×10^{-5} M, THP markedly relaxed a contraction induced by 10^{-7} M norepinephrine in rat aorta with endothelium but not in that without endothelium. At a dose of 0.02–0.5 mg, THP produced an increase in the contractile force and the perfusion flow of isolated perfused guinea pig hearts. At a higher concentration (4.5×10^{-5}–1.5×10^{-4} M), it produced a slight increase in the contractile force of the left atria in guinea pigs. This positive inotropic action of THP was inhibited by diphenhydramine (10^{-6}–5×10^{-5} M), chlorpheniramine (3×10^{-7}–3×10^{-5} M), and tripelennamine (3×10^{-7}–3×10^{-5} M) but not by propranolol (10^{-6} M), cimetidine (10^{-5} M), diltiazem (10^{-6} M), or ryanodine (10^{-8} M). THP given i.v. at 2.5 mg/kg elevated the plasma histamine level in anesthetized dogs. From these data, we conclude that THP mainly relaxed the rat aorta in the presence of endothelium and that at higher concentrations, it increased the contractile force in the cardiac muscle, probably mediated through the release of histamine.

Introduction

The anthracycline antibiotics adriamycin (ADM) and daunomycin (DM) are potent antitumor agents [4]. Their clinical use is limited by cardiotoxicity, of which two types have been identified. Acute cardiotoxicity associated with electrocardiographic changes and the appearance of abnormal left ventricular function is usually found after administration of these drugs in patients [29]. With cumulative doses of these drugs, chronic toxic cardiomyopathy may occur [5, 18]. Unverferth et al. [32] have reported that there may be only one mechanism for both types of cardiotoxicity. To lower the cardiotoxicity of the anthracycline antibiotics, great efforts were made to find new anthracyclines.

Pirarubicin [(2″*R*)-4′-*O*-tetrahydropyranyladriamycin], a derivative of ADM, was found to have a similarly potent antitumor effect but lower cardiotoxicity than ADM itself in the screening for new anthracyclines [8, 22]. Pirarubicin has been conventionally abbreviated as THP [20]. THP was also less cardiotoxic in hamsters than was ADM in electrocardiographic experiments and on observations of the myocardial structure [31]. On the other hand, it was reported that THP produced a depressor effect in anesthetized cats at doses of >0.4 mg/kg [30]. This action of THP was not inhibited by antihistamines such as cimetidine and diphenhydramine, nor was it modified by various drugs, namely, atropine, phentoramine, propranolol, hexamethonium, and reserpine [30]. Furthermore, these effects of THP were observed in pithed cats and by vagotomy [30]. These results suggest that THP depresses the blood pressure by direct action on blood vessels, not by involving the autonomic nervous system. Moreover, it has also been reported that THP (1.5×10^{-5}–1.5×10^{-6} M) produces positive chronotropic and inotropic effects in spontaneously working isolated atria of guinea pigs [30].

To clarify the mechanism of the depressor and positive inotropic effect of THP, we examined its effects on electrocardiogram (ECG), heart rate (HR), respiration, and blood pressure in anesthetized rats; on the contractile response of the thoracic aorta of rats and the left atria of guinea pigs; on the contractile response and perfusion flow of the isolated perfused hearts of guinea pigs; and on the plasma histamine level in dogs. The results show that THP depressed the blood pressure by direct action on blood vessels and that the positive inotropic action of THP may be mediated through the release of histamine.

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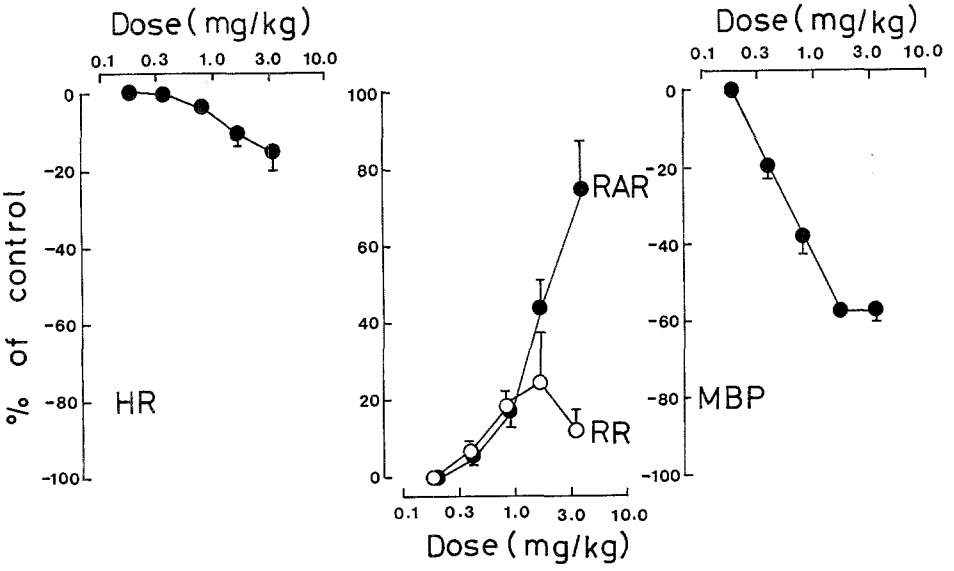

Fig. 1. Effects of THP on the heart rate (*HR*), respiration rate (*RR*), respiratory air rate (*RAR*), and mean blood pressure (*MBP*) in anesthetized rats. THP was injected into the femoral vein through a polyethylene tube. Effects of THP are expressed as percentages of change relative to the corresponding preadministration values. Data points represent the mean (± SEM) of 8–9 experiments

Materials and methods

Experimental animals. Sprague-Dawley rats (male, 250–300 g), Hartley guinea pigs (male, 250–500 g), and mongrel dogs (either sex, 7–12 kg) were used. In in vitro experiments, animals were killed by cervical dislocation and then exsanguinated, and the thoracic aorta and hearts were quickly removed.

ECG, HR, respiration, and blood pressure in anesthetized rats. Rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p.). A standard lead II ECG was monitored from needle electrodes inserted into the appropriate limbs through a bioelectric amplifier (AB-621G, Nihon Kohden), and the HR was determined from the average repetition of the R wave using a cardiotachometer (AT-601G, Nihon Kohden). Systolic, diastolic, and mean blood pressures were monitored from a cannula inserted into the right carotid artery through a pressure transducer (MPU-0.5, Nihon Kohden), and the respiration rate and respiratory air rate were obtained by means of a differential pressure transducer (TP-602T, Nihon Kohden) connected to a tracheal cannula. All signals were recorded by a multipurpose polygraph (RM-6000, Nihon Kohden). THP was injected into the femoral vein of rats through a polyethylene tube.

Experiment on the thoracic aorta in rats. The thoracic aorta was dissected and cut into several helical strips measuring 2–3 mm in width and 8–10 mm in length. In some experiments, the endothelium was removed by gently rubbing the intimal surface with a finger moistened with physiological saline solution [12]. Each muscle strip was suspended under a resting tension of 0.5 g and was equilibrated for 60 min in the bathing solution. The contractile tension of the muscle strips was recorded isometrically by a force-displacement transducer (TB-611T, Nihon Kohden) connected to a multipurpose polygraph (RM-6000, Nihon Kohden). Each preparation was examined to find out whether 10^{-6} M carbachol (CCh) would induce an almost complete (>80%) relaxation of the 10^{-7} M norepinephrine (NE)-induced contraction so as to determine the functional integrity of the endothelium. In the muscle strips from which the endothelium had been rubbed off, the 10^{-6} M CCh-induced relaxation amounted to <10% of the NE-induced contraction. The normal physiological saline solution (pH 7.4, 37° C) was composed of the following: 112 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 2 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, and 11.5 mM glucose. This solution was aerated with a mixture of 95% O_2 and 5% CO_2.

Preparation of isolated perfused guinea pig hearts. For the preparation of isolated perfused guinea pig hearts, we used Langendorff's technique as modified by Sakai et al. [27]. After a glass cannula had been filled with Krebs-Henseleit solution saturated with the 95% O_2 and 5% CO_2 mixture, each heart was mounted on a Langendorff setup. The hearts were perfused via the aorta at a perfusion pressure of 60 mmHg. The Krebs-Henseleit solution that was used as the perfusate consisted of 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and 10.6 mM glucose. For the measurement of left ventricular cavitiy pressure (LVP), a glass cannula was inserted via the left atrium into the left ventricle. The glass cannula was filled with water and connected by water-filled tubing to a pressure transducer (TP-200T, Nihon Kohden). The HR was either measured using a cardiotachometer (AT-601G, Nihon Kohden) or calculated from the LVP pulse. The first derivative of left ventricular cavity pressure (LV dP/dt) was obtained by using a differentiating circuit (EQ-601G, Nihon Kohden). Perfusion flow (PF) was measured using an electric flowmeter (MFV-2100, Nihon Kohden). The glass cannula inserted into the aorta was connected to a catheter for measurement of the PF. All parameters were recorded on a multipurpose polygraph (RM-6000, Nihon Kohden). THP was injected in a volume of 0.1 ml over a 10-s period into the aortic bulb of the heart.

Experiment in the left atria. For the mechanical experiments, the left atria of guinea pig hearts were suspended vertically in an organ bath containing 30 ml Tyrode's solution saturated with 95% O_2 – 5% CO_2 at 30° C and composed of 140 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 0.4 mM NaH_2PO_4, 12 mM $NaHCO_3$, and 5 mM glucose. Isometric contractile tension (CT) was measured using a force-displacement transducer (TB-651G, Nihon Kohden). The maximal rate of tension development (dT/dt max) was obtained by electrically differentiating contractile tension using a differentiator (ED-601G, Nihon Kohden). The resting tension was adjusted to 0.5 g and the preparation was electrically stimulated by square wave pulses of 3 ms duration at a frequency of 2 Hz and an intensity of 20% above the contractile threshold through bipolar platinum electrodes. The stimuli were delivered by an electronic stimulater (SEN-7203, Nihon Kohden) through an isolation unit (SS-302J, Nihon Kohden).

Determination of the plasma histamine level in dogs. Mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). THP was given via a venous cannula inserted into the right femoral vein. Five blood samples (6 ml) were collected via a venous cannula inserted into the left femoral vein both before and at 1, 5, 15, and 30 min after the administration of THP. Plasma histamine determinations were made according to the method of Shore et al. [28] using a fluorometer (Model 204, Hitachi).

Drugs. THP was synthesized from DM in our laboratory. THP was dissolved in physiological saline solution and in deionized water for in vivo and in vitro experiments, respectively. Drugs used in this experiment included sodium pentobarbital (Pitman-Moore), *l*-norepinephrine bitartrate (Wako), carbamilcholine chloride (carbachol, Wako), pro-

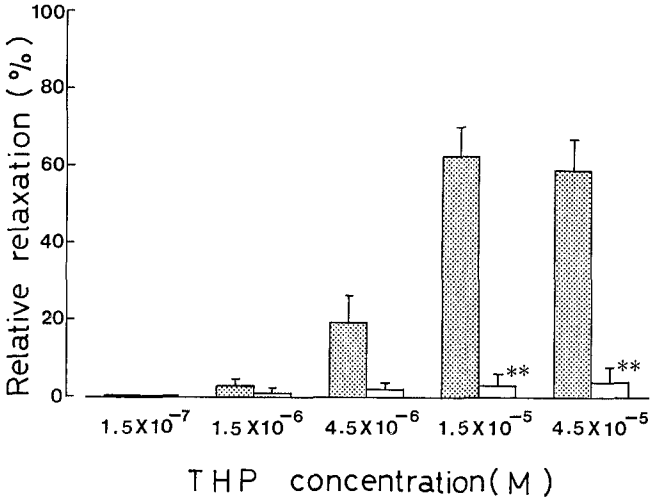

Fig. 2. Effects of THP on the norepinephrine (10^{-7} M)-induced contraction in the rat aorta with (▨) or without endothelium (□). THP was applied cumulatively in the bathing solution, producing concentrations of from 1.5×10^{-7} to 4.5×10^{-5} M. Data represent the mean (± SEM) of 5–7 experiments. **Significant difference ($P < 0.01$)

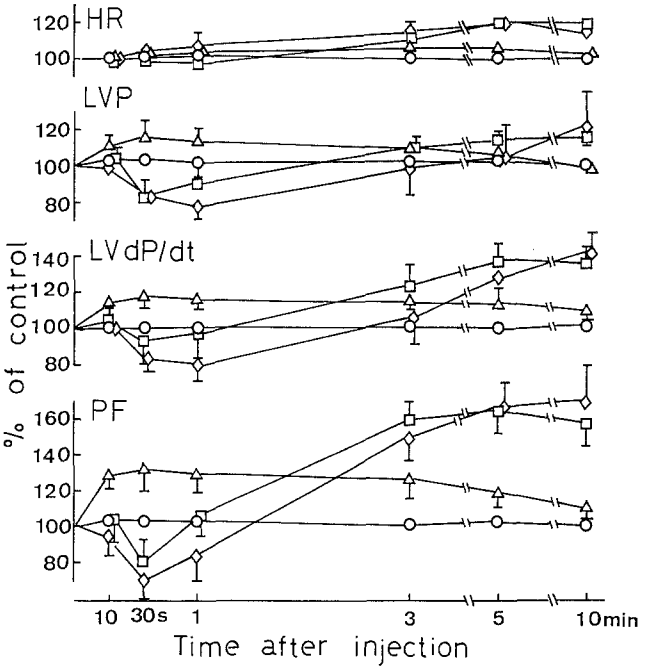

Fig. 3. Effects of THP on isolated and perfused guinea pig hearts. Single injections of THP at doses of 0.01 (○), 0.02 (△), 0.1 (□), and 0.5 mg (◇) were given. Data points represent the mean (± SEM), of 5–8 experiments. *HR*, Heart rate; *LVP*, left ventricular cavity pressure; *LV dP/dt*, first derivative of left ventricular cavity pressure; *PF*, perfusion flow

pranolol hydrochloride (Sumitomo), cimetidine (Fujisawa), diltiazem hydrochloride (Wako), diphenhydramine hydrochloride (Wako), chlorpheniramine maleate (Wako), tripelennamine hydrochloride (Sigma), and ryanodine (Wako). All drugs were dissolved in deionized water, except sodium pentobarbital, which was used in solution, and ryanodine, which was dissolved in dimethylsulfoxide (Wako) and diluted with Tyrode solution.

Statistics. Results of the experiments were expressed as mean values (± SEM). The data were analysed by Student's *t*-test and a value of $P < 0.01$ or $P < 0.05$ was considered to be significant.

Results

Effects of THP on ECG, HR, respiration, and blood pressure in anesthetized rats

Although 0.195–3.13 mg/kg THP did not affect the ECG (data not shown), the same doses produced a slight decrease in the HR and an increase in the respiration rate. On the other hand, at 0.39–3.13 mg/kg, THP produced a marked increase in the respiratory air rate, amounting to 5.5% ± 2.5%, 16.7% ± 3.9%, 43.5% ± 7.7%, and 74.1% ± 12.6% at doses of 0.39, 0.78, 1.56, and 3.13 mg/kg, respectively (Fig. 1). In the same dose range, it also markedly reduced the mean blood pressure (MBP) in a dose-dependent manner, displaying a depressor effect amounting to 19.9% ± 3.6%, 38.3% ± 4.6%, 57.5% ± 1.6%, and 57.3% ± 3.1% at doses of 0.39, 0.78, 1.56, and 3.13 mg/kg, respectively (Fig. 1).

Effects of THP on the NE-induced contraction of the aorta with or without endothelium

At concentrations ranging from 1.5×10^{-7} to 4.5×10^{-5} M, THP was cumulatively added during the sustained contraction induced by 10^{-7} M NE in the rat aorta with or without endothelium. The THP-induced relaxation of the NE-induced contractions was expressed as the percentage of decrease in tension of the precontracted state. Although 1.5×10^{-7} M THP did not relax the NE-induced contraction in aorta with endothelium, at concentrations ranging from 1.5×10^{-6} to 4.5×10^{-5} M it produced a dose-dependent relaxation that showed a maximal response at 1.5×10^{-5} M (Fig. 2). However, THP in the same concentration range had almost no effect on the contraction of NE in aorta without endothelium (Fig. 2). These results suggest that THP induced the endothelium-dependent relaxation of the NE-induced contraction.

Effects on isolated perfused guinea pig hearts

Effects of a single injection of THP on isolated and perfused guinea pig hearts were examined. At doses of 0.02–0.5 mg, THP produced a dose-dependent increase in the HR, LVP, LV dP/dt (max), and PF within 3–10 min after the injection (Fig. 3). At doses of 0.1 or 0.5 mg, it induced a transient decrease in the LVP, LV dP/dt (max), and PF (Fig. 3) immediately after the injection. In addition, increases in the LVP, LV dP/dt (max), and PF were also observed within 15–30 min after the administration of THP at doses of 0.1 or 0.5 mg (data not shown).

Effects of THP on the mechanical responses in left atria of guinea pigs

THP was applied cumulatively in the organ bath at a final concentration of from 4.5×10^{-6} to 1.5×10^{-4} M. As

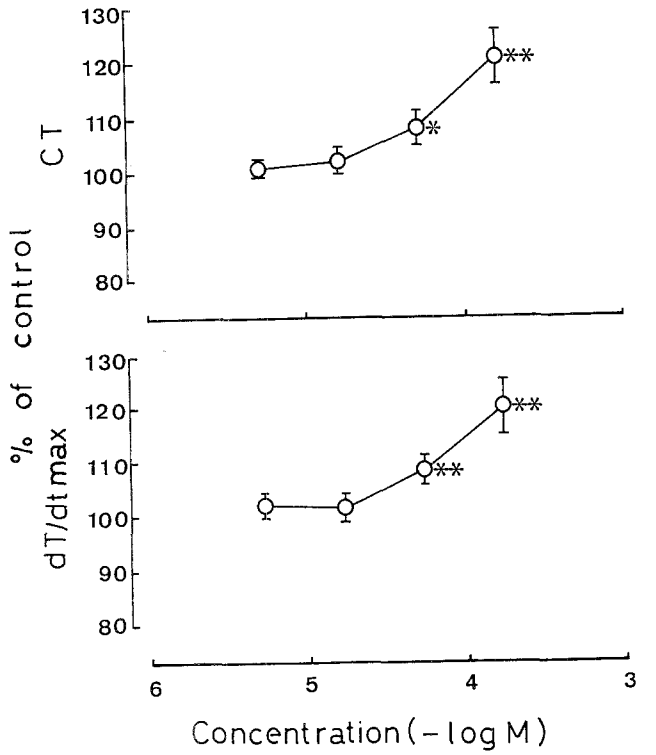

Fig. 4. Effects of THP on the isolated and electrically driven left atria of guinea pigs. THP was applied cumulatively in the organ bath at concentrations of from 4.5 × 10^{-6} to 1.5 × 10^{-4} M. Effects of THP are expressed as percentages of the initial values. Data points represent the mean (± SEM) of 9 experiments. *CT*, Contractile tension; *dT/dt max*, maximal rate of tension development. **, * Significantly different from the initial values at $P < 0.01$ and $P < 0.05$, respectively

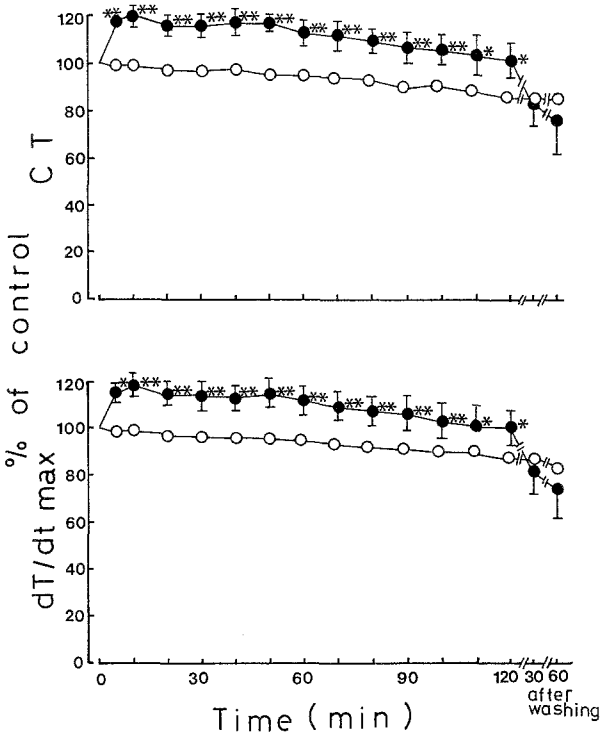

Fig. 5. Long-term effects of THP on isolated and electrically driven left atria of guinea pigs. THP (1.5 × 10^{-4} M) was applied to the incubation medium at time 0, and the effects were observed for a 2-h incubation period, following which the medium was washed and the washing effects were observed for 1 h. Data points represent the mean (± SEM) of 5 experiments. *CT*, Contractile tension; *dT/dt max*, maximal rate of tension development. **, * Significantly different from control values at $P < 0.01$ and $P < 0.05$, respectively. ○, Control; ●, 1.5 × 10^{-4} M THP

shown in Fig. 4, THP significantly increased the CT and dT/dt max in the electrically driven left atria of guinea pigs. In addition, the long-term effects of 1.5 × 10^{-4} M THP on the mechanical responses of left atria are shown in Fig. 5. THP induced a significant increase in the CT and dT/dt max immediately after the drug application; these effects continued for 2 h and were reversed by washing.

To determine the possible mechanisms responsible for the positive inotropic action of 1.5 × 10^{-4} M, preparations were pretreated with various agents. Each agent was applied 30 min prior to the addition of THP except for ryanodine (60 min). Pretreatment with 10^{-6} M propranolol, 10^{-5} M cimetidine, 10^{-6} M diltiazem, or 10^{-8} M ryanodine failed to suppress the positive inotropic action of THP (Fig. 6). However, pretreatment with the H_1-receptor antagonists diphenhydramine (10^{-5}–10^{-7} M), chlorpheniramine (3 × 10^{-5}–3 × 10^{-7} M), or tripelennamine (3 × 10^{-5}–3 × 10^{-7} M) reduced the action of THP in a concentration-dependent manner (Fig. 7).

Determination of the plasma histamine level in dogs

As shown in Fig. 8, an injection of 2.5 mg/kg THP induced an elevation of plasma histamine levels in dogs. The value measured before the injection of THP was 3.2 ± 0.5 ng/ml, which increased to a maximal level of 8.8 ± 2.9 ng/ml at 5 min after drug administration.

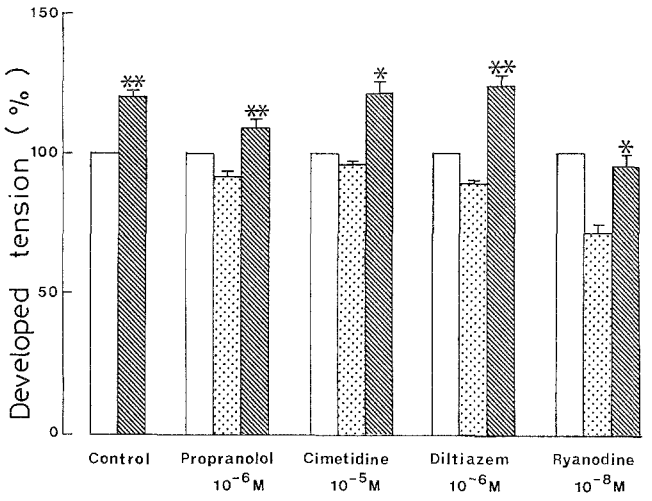

Fig. 6. Effects of various agents on the positive inotropic action of 1.5 × 10^{-4} M THP in the left atria of guinea pigs. Each agent was applied 30 min before the cumulative addition of THP. Ryanodine was applied 60 min before THP. Data points represent the mean (± SEM) of 4–5 experiments. **, * Significant difference between initial values and those obtained after application of THP at $P < 0.01$ and $P < 0.05$, respectively. □, Before antagonists; ▨, after antagonists; ▨, 10 min after THP

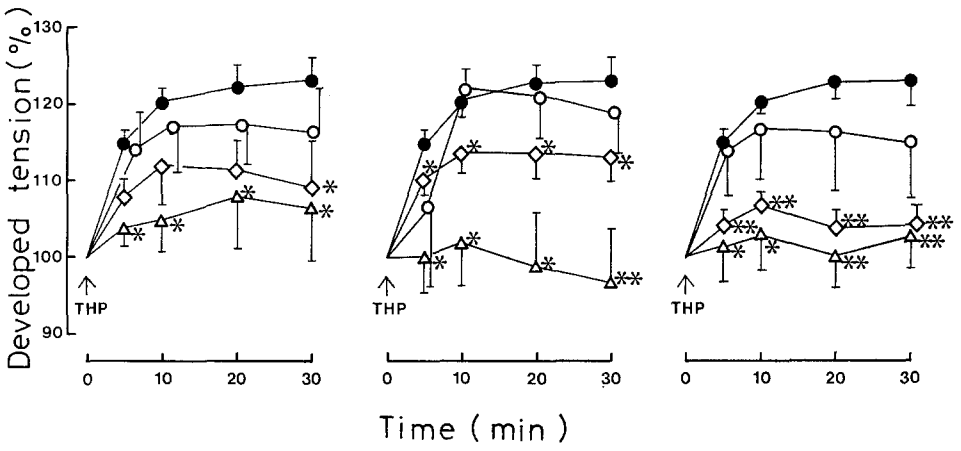

Fig. 7. Effects of diphenhydramine (*left*), chlorpheniramine (*center*), and tripelennamine (*right*) on the inotropic action of 1.5×10^{-4} M THP in the left atria of guinea pigs. Each agent was applied 30 min before the cumulative addition of THP. Data represent the mean (± SEM) of 4–6 experiments. **, * Significantly different from control values at $P < 0.01$ and $P < 0.05$, respectively. Diphenhydramine: ●, control; ○, 10^{-6} M; ◇, 10^{-5} M; △, 5×10^{-5} M. Chlorpheniramine: ●, control; ○, 3×10^{-7} M; ◇, 3×10^{-6} M; △, 3×10^{-5} M. Tripelennamine: ●, control; ○, 3×10^{-7} M; ◇, 3×10^{-6} M; △, 3×10^{-5} M

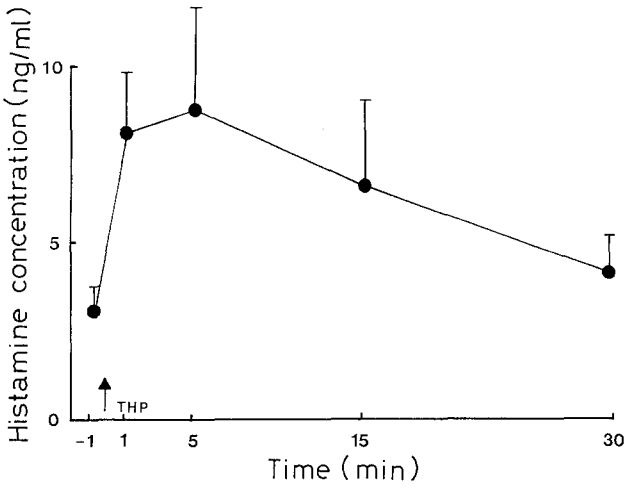

Fig. 8. Effects of i.v. injection of 2.5 mg/kg THP on the plasma histamine level in dogs. Data points represent the mean (± SEM) of 4 experiments

Discussion

In the screening of new anthracyclines, THP has been found to have a similarly potent antitumor effect but lower cardiotoxicity than ADM [8, 22, 31]. As THP displays both depressor and positive inotropic actions [30], its effects on the cardiovascular system were examined in the present study.

An injection of THP at doses of from 0.39 to 3.13 mg/kg produced a reduction in MBP that corresponded to almost the same potency needed to produce a depressor effect on cats [30] and induced dose-dependent increase in the respiratory air rate of anesthetized rats, but the same doses of THP did not affect the ECG. THP caused a marked relaxation of the NE-induced contraction in rat aorta with endothelium but not in that without endothelium. Furthermore, it increased the perfusion flow in isolated perfused guinea pig hearts. In our preliminary study, THP decreased the blood pressure and also relaxed only thoracic aorta with endothelium in guinea pigs (unpublished data). Moreover, it has been reported that the depressor effect of THP in cats was not inhibited by a pretreatment with autonomic blockades and was observed in pithed cats and by vagotomy [30].

Based on these as well as the present results, we suggest that THP produces a depressor action that is caused by an endothelium-dependent relaxation of the blood vessels. However, species differences in the cardiovascular effects of THP may be observed. In addition, previous studies have shown that treatment of dogs with DM increases ventricular contractile force responses to norepinephrine and isoproterenol but decreases maximal responsiveness, which is related to diminished myocardial functional capacity [3]. Therefore, further experiments are required to elucidate the precise mechanism of THP-induced depressor and relaxant effects in rats.

Mhatre et al. [23] have reported the effects of anthracycline antibiotics on artificially perfused or blood-perfused isolated dog hearts. In that study, higher doses of daunomycinone, a metabolite of ADM and DM, caused an increase in coronary perfusion pressure [23]. Furthermore, other investigators have reported that DM induces a contractile response and potentiates that to KCl or to BAY K 8644 in rat aorta [34, 35].

In our preliminary study, we also assessed the effects of the anthracycline antibiotics ADM, aclarubicin (ACM), and epirubicin (EPI) on the contractile response of isolated rat thoracic aorta [1]. At higher concentrations, ACM produced relaxation of rat aorta, whereas ADM and EPI did not [1]. Relaxation caused by ACM was observed in aorta with and without endothelium [1]. Furthermore, we attempted to examine the effect of 11 other anthracycline analogs on the contractile response of rat aorta with or without endothelium and found that 13-dihydropirarubicin produces endothelium-dependent relaxation (unpublished data). Accordingly, it can be said that both THP and 13-dihydropirarubicin, which contain a tetrahydropyranyl

group, among several anthracycline analogs, produce an endothelium-dependent relaxation in rat aorta.

On the other hand, several hypotheses have been put forward to explain ADM's cardiotoxicity, including its interaction with DNA [26] production of free radicals [2, 25], membrane lipid damage [9, 11], mitochondrial membrane effects [13], and modification of calcium transport [24, 33]. In addition to these hypotheses, it has been reported that histamine and catecholamine release could be related to the cardiotoxicity of ADM [6, 7]. Kobayashi et al. [17] reported that positive chronotropic and inotropic actions of ADM were blocked by propranolol in isolated perfused guinea pig hearts. In our preliminary experiments, we also examined the effects of ADM on isolated perfused guinea pig hearts and found that ADM produced positive chronotropic and inotropic action [1]; the action of ADM was inhibited by pretreatment with cimetidine or propranolol (unpublished data). These results suggest that the ADM-induced effects may be mediated through not only beta-adrenergic but also histaminic mechanisms.

In the mechanical responses of left atria, a higher concentration of THP produced a slight increase in the CT and dT/dt max. The long-term application of THP produced a slight increase in the contractile force of left atria. To determine the mechanism of THP-induced positive inotropic action, we also examined the effect of various inhibitors on the contraction of isolated left atria of guinea pigs. Although pretreatment with propranolol, cimetidine, diltiazem, or ryanodine failed to suppress the positive inotropic action of THP, pretreatment with the H_1 blockers diphenhydramine, chlorpheniramine, and tripelennamine inhibited the drug's action.

The concentrations of THP that were required to produce significant effects on guinea pig atrial muscle in vitro were substantially higher than those found in the plasma or organs of experimental animals [16] or patients [21]. However, the present results obtained in guinea pig atrial muscle could probably be useful in predicting the long-term effects of THP, because atrial muscle preparations were incubated for a relatively short period, whereas human hearts are chronically exposed to this drug. The higher concentrations of THP used in the present study are consistent with the drug concentrations used by Hagane et al. [14], who examined the effects of ADM on atrial muscle preparations at concentrations of 10^{-4} or 2×10^{-4} M.

In another experiment, we determined plasma histamine levels after the injection of THP using dogs instead of guinea pigs, because many blood samples could be sequentially obtained in the same animal and an elevation of histamine levels after the injection of ADM, DM, or other anthracyclines had been reported in dogs [10, 15]. In the present study, THP induced an elevation of plasma histamine levels. Machado et al. [19] reported that H_1 receptors mediate the positive inotropic response in the left atrium and that H_2 receptors mediate that in the right atrium and in the ventricles. These data suggest that the positive inotropic action of THP is probably mediated by histamine H_1 receptors.

In a comparison of the positive inotropic effect THP vs ADM, Hagane et al. [14] reported that ADM (10^{-4}–2×10^{-4} M) caused a transient positive inotropic effect followed by a sustained and marked negative inotropic effect in their isolated atrial muscle preparation, and similar results were obtained in our preliminary experiments with THP and other anthracyclines, but THP is much less effective on cardiac muscle than is ADM [1]. On the other hand, Herman et al. [15] and Eschalier et al. [10] reported observing an elevation in the plasma histamine level after the infusion of ADM (1.5 mg/kg) in anesthetized dogs. We obtained similar result after the injection of 2.5 mg/kg ADM in our previous experiments [1]. However, the increase in plasma histamine levels observed after the administration of THP was smaller than that caused by ADM. These data suggest that THP produces a weaker effect on histamine release than does ADM, resulting in an inotropic effect.

However, despite the hypothesis of Unverferth et al. [32], it is not certain that the acute effects of THP reliably predict its long-term toxicity. Therefore, further experiments are required to elucidate the chronic effects of THP on the cardiovascular system, since the present study examined the acute effects as a first step in clarifying the mechanism underlying the depressor and positive inotropic effect of THP using several in vitro and in vivo experiments.

In summary, the main effect of THP was to relax the vascular smooth muscle in the presence of endothelium, and only at higher concentrations did the drug produce an inotropic effect on cardiac muscle, mediated through the release of histamine.

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