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Pirarubicin-induced Endothelium-dependent Relaxation in Rat Isolated Aorta

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Abstract—The mechanism of relaxation produced by pirarubicin [(2″*R*)-4′-*O*-tetrahydropyranyladriamycin, THP] has been studied in rat isolated aorta. THP ($1{\cdot}5\times10^{-6}$–$4{\cdot}5\times10^{-5}$ M) markedly relaxed contractions induced by noradrenaline (10^{-7} M) in the aorta with endothelium, but not in that without endothelium. The relaxation induced by $1{\cdot}5\times10^{-5}$ M THP was inhibited by methylene blue (5×10^{-6} M), hydroquinone (10^{-4} M), phenidone (5×10^{-5} M), haemoglobin (10^{-6} M) and *p*-bromophenacyl bromide (5×10^{-5} M), but not by indomethacin ($2{\cdot}5\times10^{-5}$ M). The relaxation induced by THP ($1{\cdot}5\times10^{-7}$–$4{\cdot}5\times10^{-5}$ M) was inhibited by N^G-nitro-L-arginine (10^{-5} M), but enhanced by superoxide dismutase (10 units mL^{-1}) or by L-arginine (10^{-2} M). However, the THP-induced relaxation was not inhibited by various receptor antagonists such as atropine (10^{-6} M), cimetidine (10^{-5} M), diphenhydramine (3×10^{-6} M) and [D-Pro^4, D-Trp^{7,9,10}]-substance P(4–11) ($1{\cdot}5\times10^{-6}$ M). In fifteen anthracycline analogues, THP and 13-dihydropirarubicin (both with a tetrahydropyranyl group) produced endothelium-dependent relaxations. These results suggest that the THP-induced relaxation which is probably mediated by endothelium-derived relaxing factor (EDRF) was not produced by an activation of muscarine, histamine H_1 or H_2, or substance P receptor, and further that the tetrahydropyranyl group must play an important role in the THP-induced relaxation.

Pirarubicin, (2″*R*)-4′-*O*-tetrahydropyranyladriamycin, is a derivative of the anthracycline antibiotic doxorubicin, and a potent antitumour agent (Matsushita et al 1985). Pirarubicin has been conventionally abbreviated as THP (Majima 1982). In cardiovascular studies THP has been shown to have a depressor effect in anaesthetized cats and rats (Tone et al 1986). The effect in the cat was not influenced by pithing, by vagotomy or by treatment with antihistamines such as cimetidine or diphenhydramine, nor was it modified by atropine, phentolamine, propranolol, hexamethonium or reserpine (Tone et al 1986). These results suggested that THP decreased the blood pressure by direct action on the blood vessels, not by involving the autonomic nervous system or autacoids. In a previous study, we found that THP increased perfusion flow of the isolated and perfused guinea-pig heart, relaxed noradrenaline-induced contraction of rat aorta, and confirmed the previous result that THP directly relaxed the blood vessels (Hirano et al 1991). On the other hand, endothelium-dependent relaxation of blood vessels caused by acetylcholine and a large number of other substances has been reported (Furchgott & Zawadzki 1980; Furchgott 1981, 1984; Cherry et al 1982; Cocks & Angus 1983; Van de Voorde & Leusen 1983). It has been demonstrated that this endothelium-dependent relaxant effect is mediated by endothelium-derived relaxing factor (EDRF) in response to stimulation of agonist-specific receptors (Furchgott & Zawadzki 1980; Griffith et al 1984; Cocks et al 1985). Furthermore, it has been confirmed that EDRF is identical with nitric oxide (NO) (Palmer et al 1987) and formed from L-arginine (Palmer et al 1988). Therefore, in the present experiments, we examined the effects of THP and other anthracycline analogues on the contractile response of rat aorta with or without endothelium, the effect of inhibitors relating to synthesis or release of EDRF, and the effect of various receptor antagonists on the THP-induced relaxation in rat aorta. We also examined the effects of N^G-nitro-L-arginine (L-NNA), an inhibitor of cytosolic NO formation from L-arginine (Moore et al 1990; Mülsch & Busse 1990), and of superoxide dimustase (SOD), an inactivator of superoxide anion (O_2^-) formed from O_2 (Gryglewski et al 1986), and of L-arginine, a substrate of NO (Moore et al 1990, Mülsch & Busse 1990), on the THP-induced relaxation in the aorta.

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Materials and Methods

Rat aortic strips

Male Sprague-Dawley rats, 250–300 g, were killed by a blow on the head and exsanguinated. The removed thoracic aortas were placed in physiological saline solution (PSS) and carefully cleaned of surrounding connective tissue. The normal PSS contained (mM): NaCl 118·3, KCl 4·7, $CaCl_2$ 2·0, $MgSO_4$ 1·2, $NaHCO_3$ 25·0, KH_2PO_4 1·2, calcium EDTA 0·026 and glucose 11·1. The thoracic aorta was cut into several helical strips, 2–3 mm wide and 8–10 mm long. In some experiments, the endothelium was removed by gently rubbing the intimal surface with a finger moistened with PSS (Furchgott & Zawadzki 1980). Each muscle strip with or without endothelium was attached to a holder under a resting tension of 0·5 g and equilibrated for 60 min in a 10 mL organ bath filled with PSS (37°C, pH 7·4), bubbled with 95% O_2–5% CO_2. The contractile tension of muscle strip was recorded isometrically with a force transducer (TB-611T, Nihon Kohden, Tokyo, Japan) connected to a multipurpose polygraph (RM-6000, Nihon Kohden). Each preparation was checked to confirm 10^{-6} M carbachol induced an almost

complete (more than 80%) relaxation of the 10^{-7} M noradrenaline-induced contraction in order to determine the functional integrity of the endothelium. In the muscle strips from which the endothelium had been removed, the 10^{-6} M carbachol-induced relaxation was less than 10% of the noradrenaline-induced contraction.

Aorta with or without endothelium

In the aorta with endothelium, THP and other anthracycline analogues were cumulatively applied during the sustained contraction induced by noradrenaline (10^{-7} M) or KCl (55·9 mM). In another series of experiments, THP and other anthracycline analogues were also applied during the sustained contraction induced by noradrenaline (10^{-7} M) in the aorta without endothelium.

Application of inhibitors or activator relating to synthesis or release of EDRF

Effects of the inhibitors relating to synthesis or release of EDRF on THP- or carbachol-induced relaxation in the aorta with endothelium were also examined as described by Griffith et al (1984) and Nagase et al (1987). In muscle strips precontracted by noradrenaline (10^{-7} M), carbachol (3×10^{-7} M) or THP ($1{\cdot}5 \times 10^{-5}$ M) was applied to the sustained contraction. When the carbachol or THP-induced relaxation reached a steady level, various inhibitors were applied and the recovery of muscle tension caused by each was measured. In another series of experiments, inhibitor was added 5 min before the addition of noradrenaline. THP ($1{\cdot}5 \times 10^{-5}$ M) was applied 10 min after the addition of noradrenaline.

Effects of agents related to NO synthesis on THP-induced relaxation in the aorta were also examined. L-NNA (10^{-5} M) was applied 5 min before the addition of noradrenaline (10^{-7} M), and THP ($10^{-7} - 3 \times 10^{-5}$ M) was applied 10 min after the addition of noradrenaline. On the other hand, SOD (10 units mL^{-1}) or L-arginine (10^{-2} M) was applied 10 min after the addition of noradrenaline, and THP was cumulatively applied 5 min after the addition of noradrenaline.

Application of receptor antagonists

Effects of receptor antagonists on THP-induced relaxation in the aorta with endothelium were also examined. Each antagonist was applied 5 min before the addition of noradrenaline (10^{-7} M), and THP ($10^{-7} - 3 \times 10^{-5}$ M) was applied 10 min after the addition of noradrenaline.

Drugs

THP, synthesized from daunorubicin in our laboratory, was dissolved in deionized water. Other anthracycline analogues, aclarubicin (aclacinomycin A) hydrochloride, doxorubicin (adriamycin) hydrochloride, epirubicin hydrochloride, aklavin, aclacinomycin B, 2-hydroxyaclacinomycin A, betaclamycin A, betaclamycin T, epelmycin B, oxaunomycin, 13-dihydropirarubicin, 4-*O*-methylbetaclamycin T, aklavinone, and baumycin A1 were dissolved in deionized water or in dimethylsulphoxide (DMSO, Wako, Tokyo, Japan). Final concentration of DMSO was 0·4% or less. These anthracycline analogues were supplied by our laboratory except that doxorubicin and epirubicin were obtained from the Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan). Other drugs used in this experiment were (−)-noradrenaline bitartrate (Wako), carbamylcholine chloride (carbachol, Wako), indomethacin (Sigma, St Louis, MO), phenidone (Wako), haemoglobin (Sigma), hydroquinone (Wako), *p*-bromophenacyl bromide (Dojin, Kumamoto, Japan), atropine sulphate (Tanabe, Osaka, Japan), methylene blue trihydrate (Wako), N^G-nitro-L-arginine (Aldrich, Milwaukee, WI), L-arginine monohydrochloride (Wako), superoxide dismutase (Wako), cimetidine (Sumitomo, Osaka, Japan), diphenhydramine hydrochloride (Wako) and [D-Pro^4, D-Trp^{7,9,10}]-substance P(4-11)(Peninsula, Belmont, CA). Indomethacin and *p*-bromophenacyl bromide were dissolved in DMSO and diluted with PSS to yield a final concentration of 0·1%. Other drugs were dissolved in deionized water.

Statistical analysis

Results of the experiments were expressed as mean ± s.e.m. The data were analysed by Student's unpaired *t*-test and $P < 0{\cdot}05$ was defined as significant.

Results

Effects of THP on noradrenaline- or KCl-induced contraction in rat aorta

Although $1{\cdot}5 \times 10^{-7}$ M THP did not relax the aorta precontracted with 10^{-7} M noradrenaline, THP ($1{\cdot}5 \times 10^{-6} - 4{\cdot}5 \times 10^{-5}$ M) produced a concentration-dependent relaxation. Maximum response to $1{\cdot}5 \times 10^{-5}$ M THP was 62·9 ± 7·2% of the 10^{-7} M noradrenaline-induced response (Fig. 1A). With the same range of concentrations, THP was much less effective in relaxing the KCl-induced contraction, as $1{\cdot}5 \times 10^{-5}$ M THP relaxed only 6·3 ± 1·2% of the KCl-induced contraction (Fig. 1B).

In the aorta without endothelium, the application of THP ($1{\cdot}5 \times 10^{-7} - 4{\cdot}5 \times 10^{-5}$ M) to the sustained contraction induced by noradrenaline (10^{-7} M) had no effect (Fig. 1A). The concentration-relaxation curves for THP were obtained from the experiments of the aorta with and without endothelium as shown in Fig. 1B.

Effects of inhibitors on the THP-induced endothelium-dependent relaxation

In this experiment, application of 0·1% DMSO or of the inhibitors mentioned below had no effect on the basal tension of the aorta. As shown in Fig. 2, methylene blue (5×10^{-6} M), hydroquinone (10^{-4} M), phenidone (5×10^{-5} M), haemoglobin (10^{-6} M) and *p*-bromophenacyl bromide (5×10^{-5} M) significantly reversed the relaxing effect of THP ($1{\cdot}5 \times 10^{-5}$ M) or carbachol (3×10^{-7} M). Indomethacin ($2{\cdot}5 \times 10^{-5}$ M) did not affect the relaxation induced by THP or carbachol.

In another series of experiments, the effect of pretreatment with methylene blue (5×10^{-6} M) on the THP-induced relaxation was examined. The application of THP ($1{\cdot}5 \times 10^{-5}$ M) slightly relaxed the aorta precontracted with noradrenaline (data not shown).

Effects of agents related to NO synthesis on the THP-induced endothelium-dependent relaxation

As shown in Fig. 3, pretreatment with L-NNA (10^{-5} M) caused a complete inhibition of relaxation induced by THP

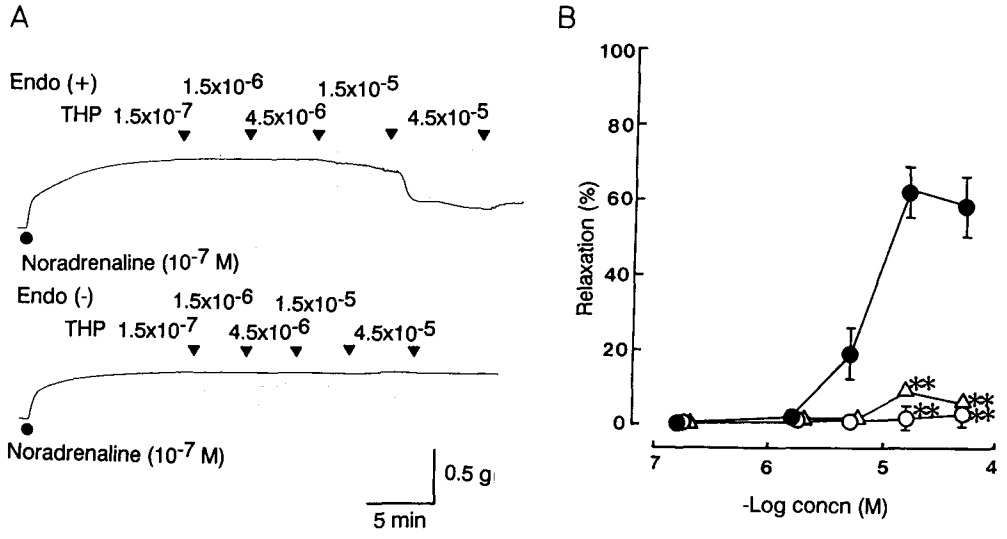


FIG. 1. Effects of THP on the 10^{-7} M noradrenaline-induced contraction in the rat aorta with [Endo(+), ●] or without endothelium [Endo(−), ○] or on the 55·9 mM KCl-induced contraction in the aorta with endothelium (△). THP was applied in the bathing solution at concentrations from $1{\cdot}5 \times 10^{-7}$ to $4{\cdot}5 \times 10^{-5}$ M. A: Typical recording of mechanical response from several experiments. B: Concentration-relaxation curves for THP. Data points are mean ± s.e.m of 4 to 7 experiments. In some cases, the s.e.m. was smaller than the symbol. $^{**}P < 0{\cdot}01$ compared with the effect of THP on the noradrenaline-induced contraction in the aorta with endothelium.

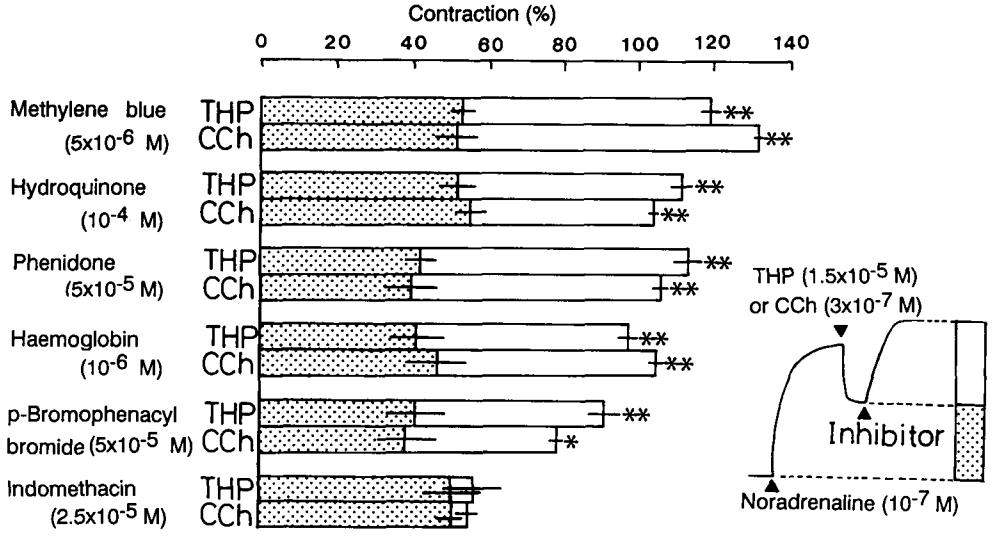


FIG. 2. Effects of inhibitors on the THP- or carbachol-induced relaxation in rat aorta with endothelium. During the sustained contraction induced by 10^{-7} M noradrenaline, THP ($1{\cdot}5 \times 10^{-5}$ M) or carbachol (3×10^{-7} M) was applied. When the THP- or carbachol-induced relaxation reached a steady level (dotted column), an inhibitor was applied and recovery of the muscle contraction was measured (open column). One hundred percent represents the noradrenaline-induced contraction before the application of THP or carbachol, and 0% represents the resting tension of the aorta. Mean ± s.e.m. of 4 to 9 experiments are shown. $^{**}P < 0{\cdot}01$, $^{*}P < 0{\cdot}05$ compared with the muscle contraction which had previously been inhibited by THP or carbachol.

($1{\cdot}5 \times 10^{-7}$–$4{\cdot}5 \times 10^{-5}$ M). THP, $1{\cdot}5 \times 10^{-5}$ or $4{\cdot}5 \times 10^{-5}$ M, relaxed only 0·1 ± 0·1%, or 3·8 ± 0·9% of the aorta pretreated with L-NNA, respectively. On the other hand, pretreatment with SOD (10 units mL^{-1}) or L-arginine (10^{-2} M) caused an increase of relaxation induced by THP ($1{\cdot}5 \times 10^{-7}$–$4{\cdot}5 \times 10^{-5}$ M) (Fig. 3).

Effects of other anthracycline analogues on noradrenaline or KCl-induced contraction in rat aorta

In this experiment, application of 0·4% DMSO had no effect on the contraction to noradrenaline. Table 1 and Fig. 4 show respectively the chemical structures of THP and other anthracycline analogues, and the effects on noradrenaline- or KCl-induced contractions in rat aorta. In fifteen anthracycline analogues, both THP and 13-dihydropirarubicin produced the endothelium-dependent relaxation in the aorta precontracted with noradrenaline. Doxorubicin, epirubicin or baumycin A1 had no effect on any noradrenaline- or KCl-induced contractions. At higher concentrations, aclarubicin (aclacinomycin A), aclacinomycin B, aklavin, aklavinone,

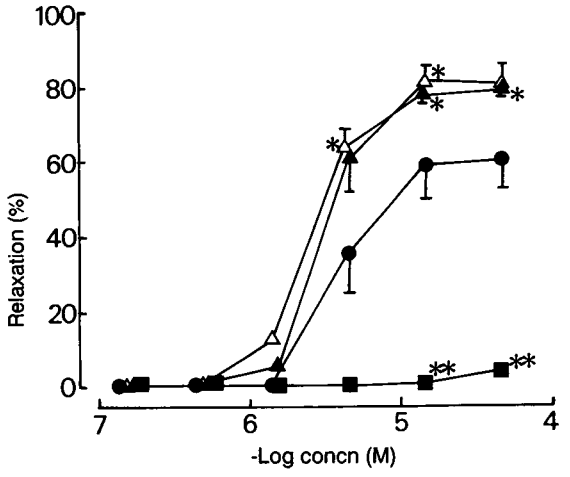


FIG. 3. Effects of agents relating to a NO synthesis on the THP-induced endothelium-dependent relaxation. N^G-Nitro-L-arginine (L-NNA, 10^{-5} M) was applied 5 min before the addition of noradrenaline (10^{-7} M), and THP ($10^{-7}-3\times10^{-5}$ M) was applied 10 min after the addition of noradrenaline. Superoxide dismutase (SOD, 10 units mL^{-1}) or L-arginine (10^{-2} M) was applied 10 min after the addition of noradrenaline, and THP was cumulatively applied after the addition of noradrenaline. ●, Control; ■, L-NNA; △, SOD; ▲, L-arginine. Data points are mean ± s.e.m. of 5 to 6 experiments. $^{**}P<0.01$, $^{*}P<0.05$ compared with control.

betaclamycin T, 4-*O*-methylbetaclamycin T or oxaunomycin all relaxed contractions induced by noradrenaline in the aorta both with and without endothelium. However, these analogues produced either a slight relaxation or no effect in the aorta precontracted with KCl. 2-Hydroxyaclacinomycin A or epelmycin B caused a slight relaxation in the aorta without endothelium. Betaclamycin A produced a moderate relaxation in the aorta only without endothelium.

Effects of various receptor antagonists on the THP-induced endothelium-dependent relaxation

We examined the effects of receptor antagonists, namely atropine (10^{-6} M), cimetidine (10^{-5} M), diphenhydramine (3×10^{-6} M) and [D-Pro^4,D-Trp^{7,9,10}]-substance P (4-11) ($1{\cdot}5\times10^{-6}$ M) on the relaxation induced by THP ($1{\cdot}5\times10^{7}-4{\cdot}5\times10^{-5}$ M). The relaxation caused by THP was not affected by pretreatment with each of the above receptor-antagonists (data not shown).

Discussion

It was reported that THP produced a depressor effect in anaesthetized cats, which was probably caused by a direct action of THP on the blood vessels (Tone et al 1986). In the previous study (Hirano et al 1991), we reported that THP decreased blood pressure of anaesthetized rats in a dose-dependent manner and increased perfusion flow in the guinea-pig isolated and perfused heart. Moreover, THP relaxed contractions induced by noradrenaline in the rat aorta with endothelium (Hirano et al 1991). From this data, it has been suggested that the THP-induced depression is caused directly by a relaxation of the blood vessels. In the present paper, THP markedly relaxed noradrenaline-induced contraction in the aorta with endothelium, but had no effect on that without endothelium. In addition, THP may not have α-blocking properties, since pretreatment of THP ($1{\cdot}5\times10^{-6}-4{\cdot}5\times10^{-5}$ M) did not inhibit the contraction induced by 10^{-7} M noradrenaline in rat isolated aorta without endothelium (unpublished data). These results suggest that THP produced the endothelium-dependent relaxation.

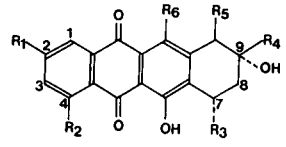
In another series of experiments, we found that the THP- or carbachol-induced relaxation was inhibited by several inhibitors, methylene blue, an inhibitor of guanylate cyclase (Gruetter et al 1981), hydroquinone and phenidone, antioxidants or radical scavengers (Griffith et al 1984), haemoglobin, an EDRF-binding protein (Martin et al 1985) and *p*-bromophenacyl bromide, an inhibitor of phospholipase A_2 (Furchgott 1984). Furchgott & Zawadzki (1980) have demonstrated that acetylcholine produces relaxation of the aorta which is mediated by the endothelium, and that the relaxation was inhibited by the inhibitors mentioned above. Nagase et al (1987) reported that the relaxing effect of carbachol was also inhibited by these same inhibitors. On the other hand, it is known that the vascular endothelial cells releases prostaglandin I_2 (PGI_2), as a relaxing substance (Toda 1980; Okamura et al 1989), and that synthesis of PGI_2 is inhibited by indomethacin, an inhibitor of cyclo-oxygenase (Skidgel & Printz 1987). The THP- or carbachol-induced relaxation was not inhibited by treatment with indomethacin. From these reports and our present data, it seems that THP, as well as the cholinergic agents, probably relaxes the vascular smooth muscle involving the synthesis or release of EDRF, but not of PGI_2. Moreover, both EDRF (Murakami et al 1985) and nitroxide compounds (Lincoln 1982; Karaki et al 1984; Murakami et al 1987) which increase cGMP content in the vascular smooth muscle by stimulating guanylate cyclase, markedly relaxed the noradrenaline-induced contraction, but not the KCl-induced contraction. 8-Bromo-cGMP (Lincoln 1982), which permeates the cell membrane, also showed a similar effect on both these contractions. The hypothesis that THP relaxes the smooth muscle in relation to EDRF is supported by the present data that THP markedly relaxed the noradrenaline-induced contraction but not the KCl-induced contraction.

In several mammalian cell types there exists an oxidative L-arginine pathway, which leads to the formation of L-citrulline and NO (Iyengar et al 1987; Amber et al 1988; Schmidt et al 1988; Palmer et al 1988). NO in turn participates in endothelium-dependent relaxation, and evidence has been presented that EDRF is identical with NO (Palmer et al 1987). Recently, L-NNA was found to inhibit NO biosynthesis by vascular endothelial cells (Moore et al 1990; Mülsch & Busse 1990). Furthermore, it has been reported that O_2^- inactivates the EDRF released from superfused aortic smooth muscle strips, and that EDRF is protected from breakdown by SOD (Gryglewski et al 1986). In order to clarify whether the EDRF produced by THP-induced endothelium-dependent relaxation is truly identical with NO, we examined the effects of L-NNA, SOD, and L-arginine in rat isolated aorta. THP-induced relaxation was completely blocked by pretreatment with L-NNA. However, the relaxation caused by THP was enhanced by pretreatment with SOD or L-arginine. These results suggest that the EDRF released or synthesized from endothelial cells in the relaxation caused by THP is indeed identical with NO.

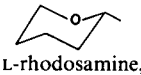
Recently, Wakabayashi et al (1989, 1990) showed that daunorubicin, an anthracycline antibiotic, increases contractile response to KCl and BAY K 8644 in rat aorta. In the present study, we examined the effects of other anthracycline analogues on rat aorta to clarify the structure-activity relationship of anthracyclines. At higher concentrations, aclarubicin (aclacinomycin A), aclacinomycin B, aklavin, aklavinone, betaclamycin T, 4-*O*-methylbetaclamycin T, oxaunomycin, betaclamycin A, 2-hydroxyaclacinomycin A or epelmycin B all produced relaxation in the aorta without endothelium. Doxorubicin, epirubicin, and baumycin Al had no effect; however, 13-dihydropirarubicin produced an endothelium-dependent relaxation in rat aorta. Accordingly, it can be said that only THP and 13-dihydropirarubicin which have the tetrahydropyranyl group, of all the above anthracycline analogues show an endothelium-dependent relaxation in rat aorta. From these results, it is suggested that the tetrahydropyranyl group in anthracycline analogues plays an important role in producing endothelium-dependent relaxation of rat aorta.

Some of the agents that produced endothelium-dependent relaxation of blood vessels are receptor agonists, and the relaxation caused by these agents may be limited to certain species or blood vessels (Furchgott & Vanhoutte 1989). With

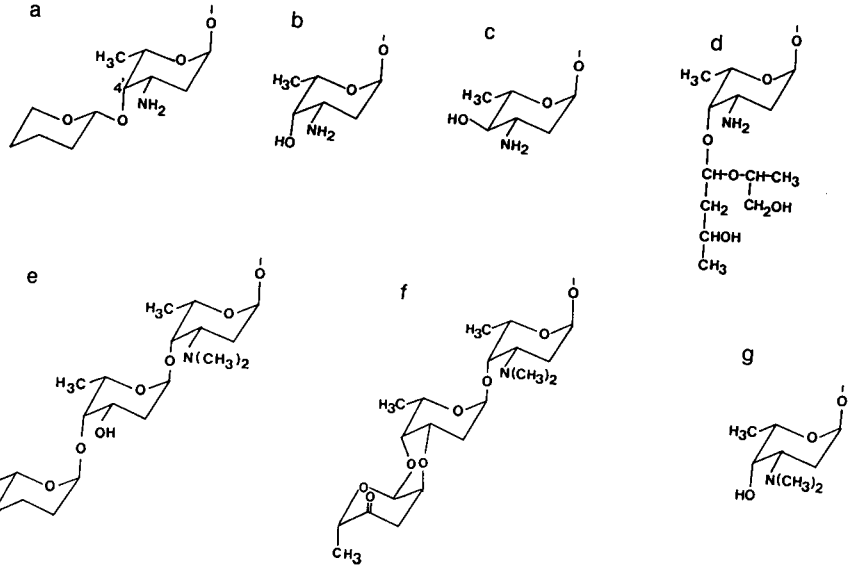
Table 1. Chemical structures of anthracycline analogues.



Analogues	R1	R2	R3	R4	R5	R6
Pirarubicin	H	OCH_3	4′-substituted[a] DN	CH_2COCH_2OH	H	OH
13-Dihydropirarubicin	H	OCH_3	4′-substituted[a] DN	$CH_2CH(OH)CH_2OH$	H	OH
Doxorubicin	H	OCH_3	DN[b]	CH_2COCH_2OH	H	OH
Epirubicin	H	OCH_3	epiDN[c]	CH_2COCH_2OH	H	OH
Baumycin Al	H	OCH_3	4′-substituted[d] DN	CH_2COCH_3	H	OH
Aclarubicin (aclacinomycin A)	H	OH	RN-dF-Cin A[e]	CH_2CH_3	$COOCH_3$	H
Aclacinomycin B	H	OH	RN-dF-Cin B[f]	CH_2CH_3	$COOCH_3$	H
Aklavin	H	OH	RN[g]	CH_2CH_3	$COOCH_3$	H
Aklavinone	H	OH	OH	CH_2CH_3	$COOCH_3$	H
2-Hydroxyaclacinomycin A	OH	OH	RN-dF-Cin A	CH_2CH_3	$COOCH_3$	H
Epelmycin B	H	OH	RN-dF-Cin B	CH_2CH_3	$COOCH_3$	OH
Betaclamycin A	H	OH	RN-dF-Cin A	CH_2CH_3	OH	OH
Betaclamycin T	H	OH	RN	CH_2CH_3	OH	OH
4-*O*-Methylbetaclamycin T	H	OCH_3	RN	CH_2CH_3	OH	OH
Oxaunomycin	H	OH	DN	CH_2CH_3	OH	OH

Tetrahydropyranyl group: 

DN: L-daunosamine, RN: L-rhodosamine, dF: 2-deoxy-L-fucose. Cin A: L-cinerulose A, Cin B: L-cinerulose B.



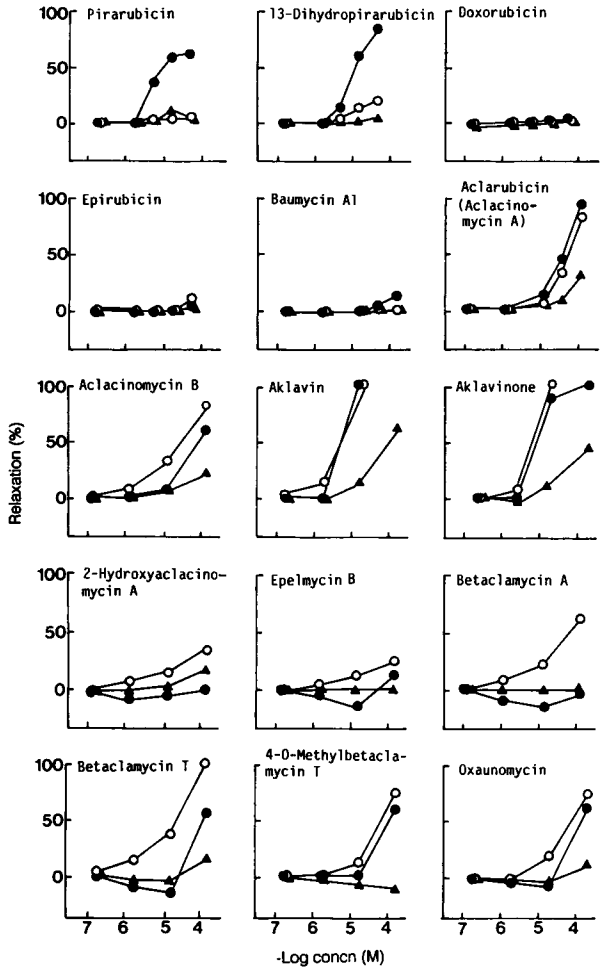


FIG. 4. Effects of anthracycline analogues on the 10^{-7} M noradrenaline-induced contraction in the rat aorta with (●) or without endothelium (○) or on the 55·9 mM KCl-induced contraction in the aorta with endothelium (▲). Anthracycline analogues were cumulatively applied during the sustained contraction induced by noradrenaline or KCl. Data points are mean of 2 to 7 experiments.

rat isolated thoracic aorta the endothelium-dependent relaxation has been reported with acetylcholine (Winquist et al 1985), carbachol (Nagase et al 1987), histamine (Van de Voorde & Leusen 1983), substance P (Jeremy & Dandona 1989) and vasoactive intestinal peptide (Davies & Williams 1983). Furthermore, in the guinea-pig isolated atria, we previously reported that THP at higher concentrations induced a positive inotropic action which may be mediated by release of histamine (Hirano et al 1991). In the present study, therefore, we have examined the possible involvement of a receptor in the activation of endothelial cells by THP. The THP-induced relaxation was not inhibited by receptor antagonists such as atropine, cimetidine, diphenhydramine or [D-Pro^4,D-Trp^{7,9,10}]-substance P (4-11). These results suggest that the THP-induced relaxation was not mediated by muscarine, histamine H_1 or H_2, or substance P receptors. However, the present results do not explain how THP releases or synthesizes EDRF in the endothelial cells. Thus, further experiments are required to elucidate the precise mechanism of the THP-induced endothelium-dependent relaxation.

It is suggested that the THP-induced relaxation, probably mediated by EDRF, did not involve muscarine, histamine H_1 or H_2, or substance P receptors, and that the tetrahydropyranyl group plays an important role in the THP-induced relaxation.

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