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Potentiating Effect of Daunorubicin on Vasocontractile Responses to KCl and BAY K 8644 in Rat Aorta

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Abstract—The effects of daunorubicin on vasocontraction by several agonists have been investigated on isolated aortic strips from rats. Pretreatment of the strips with daunorubicin (17·7 μM) potentiated the contractile response to low concentrations of KCl or to BAY K 8644 but not to phenylephrine or clonidine. The maximal contractile response to KCl was not affected by the pretreatment while that to BAY K 8644 was increased. The potentiated response to KCl could be eliminated by addition of nifedipine (1 μM) or use of a calcium-free solution. The maximal contractile response to BAY K 8644 was greatly increased by partial depolarization with KCl (10 mM, final concn) in the control solution but only slightly increased by the partial depolarization in the solution with daunorubicin. These results suggest that daunorubicin facilitates activation of the voltage-dependent calcium channel and increases the contractile responses to KCl and BAY K 8644 in rat aorta.

The uses of the anthracycline antibiotic daunorubicin in clinical oncology are limited by its toxic effects, mainly myelodepression and cardiotoxicity. Previous papers have reported that daunomycinone, a metabolite of daunorubicin and doxorubicin, increases coronary perfusion pressure of isolated hearts from dogs (Mhatre et al 1971) and that intravenous administration of doxorubicin increases arterial blood pressure and decreases renal blood flow in rats (Tvete et al 1982). On the other hand, doxorubicin has been reported to show an acute hypotensive effect via release of histamine in dogs (Herman et al 1978) and chronic pretreatment of rats with doxorubicin has been shown to attenuate the contractile response to noradrenaline in isolated aortic strips (Dalske & Hardy 1988). In comparison with these in-vivo studies, there is little information on the in-vitro action of anthracycline drugs on vascular smooth muscle. Our recent report has shown that daunorubicin (> 35·5 μM) directly stimulates vascular smooth muscle and induces a contractile response in rat aorta (Wakabayashi et al 1989b). However, the mechanism of this action is unclear. The present study examines the effects of a lower concentration of daunorubicin (17·7 μM) on contractile responses to several agonists in isolated aortic strips from rats. The daunorubicin concentration used was lower than those producing cardiotoxicity in the previous in-vitro experiment using isolated heart preparations from rats (Burns & Dow 1980).

Materials and Methods

Measurement of rat aortic contraction

Male wistar rats, 6–8 months, were killed by stunning and exsanguination. The thoracic aortas were removed and placed in Krebs-Ringer bicarbonate solution (composition, mM: NaCl 118; KCl 4·7; $CaCl_2$ 2·5; KH_2PO_4 1·2; $MgSO_4$ 1·2; glucose 10 and $NaHCO_3$ 25). After removal of excess fat and connective tissue, helical strips (approximately 2 × 15 mm) were prepared. Each strip was then suspended vertically between hooks in a 10 mL organ chamber filled with the above solution (37 C, pH 7·4), bubbled with 95% O_2–5% CO_2, and attached to a force transducer connected to a Nihon Kohden polygraph. After 1 h of equilibration with a resting tension of 1 g, changes in the isometric force were recorded. In all preparations, the endothelium was removed by gentle abrasion of the intimal surface with ultrafine sandpaper (Nippon Coated Abrasive C-1000). Denudation of the endothelium was confirmed functionally by the disappearance of the 10 μM acetylcholine-induced relaxing response of the 0·1 μM noradrenaline precontracted vessel at the end of the experiments (Wakabayashi et al 1987). The contractile response was expressed in terms of the percentage of the 80 mM KCl-induced contractile force in each strip. The "control" and "daunorubicin-treated" data were obtained on the same preparations from the same rat. The concentration-response curves of four contractile agonists (KCl, BAY K 8644, phenylephrine, clonidine) were all reproducible in the absence of daunorubicin.

Drugs

Drugs used were daunorubicin hydrochloride (Meiji Seika), BAY K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) (Wako), nifedipine (Sigma), phenylephrine hydrochloride (Sigma), noradrenaline hydrochloride (Sigma) and acetylcholine chloride (Wako). BAY K 8644 and nifedipine were dissolved in ethanol to make up each stock solution of 1 mM. The final concentrations of ethanol were below 0·1%, which did not affect the contraction by KCl. All experiments with BAY K 8644 and nifedipine were conducted in the dark. The concentration of each drug was expressed as the final concentration in the organ bath.

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Data analysis

Mean values of contractile force were arithmetically calculated and those of EC50 (the concentration of agonist producing a half-maximal response) were geometrically calculated. The data were expressed as mean ± s.e. The dispersion of EC50 values is expressed in linear terms. The EC50 value was determined graphically from individual concentration-response curves. Statistical analyses were by Student's paired *t*-test with $P < 0.05$ being defined as significant.

Results

Effect of daunorubicin on submaximal contractile responses to KCl (15 mM)

Pretreatment of aortic strips with daunorubicin (17·7 μM), which did not affect the basal tension of the strips or occasionally caused a slight vasocontraction (a force less than 50 mg), markedly potentiated the KCl (15 mM)-induced contractile response. Such contractile response to KCl in the presence of daunorubicin was eliminated by the addition of nifedipine, a calcium channel antagonist, or by the replacement of the solution with a calcium-free one (Fig. 1A, B, C).

Effect of daunorubicin on maximal contractile response to BAY K 8644

Contractile responses to BAY K 8644, an activator of the voltage-dependent calcium channel, were also markedly potentiated by pretreatment of the strips with daunorubicin. When KCl (10 mM) was added to the organ bath after the maximal contractile response had been elicited by BAY K 8644 (1 μM), only a small contraction was produced in the strips pretreated with daunorubicin, whereas a large increase occurred in the untreated strips (Fig. 1D, E).

Effect of daunorubicin on concentration-response relationships of KCl- and BAY K 8644-induced contractions

Daunorubicin significantly potentiated the response to KCl (≦30 mM). The EC50 value for KCl was significantly reduced by daunorubicin pretreatment compared to the 0·9% NaCl (saline)-pretreated control [12·9 (+1·6 to −1·4) mM (daunorubicin), 20·7 (+1·5 to −1·4) mM (control) ($P < 0.05$)]. However, the maximal response to KCl was not affected by pretreatment with this drug (Fig. 2).

Daunorubicin markedly potentiated the contractile responses to all concentrations of BAY K 8644. The EC50 value for BAY K 8644 was significantly reduced by daunorubicin pretreatment compared with the saline-pretreated control [1·45 (+0·55 to −0·40) × 10^{-8} M (daunorubicin), 7·41 (+1·50 to −1·24) × 10^{-8} M (control) ($P < 0.05$)]. Daunorubicin also significantly potentiated the maximal reponse to BAY K 8644 (Fig. 2).

Effects of daunorubicin on concentration-response relationships of phenylephrine- and clonidine-contractions

Pretreatment of aortic strips with daunorubicin did not affect the concentration-response relationship of phenylephrine-contraction (Fig. 3). Also, pretreatment with daunorubicin did not affect that of clonidine contraction (Fig. 3).

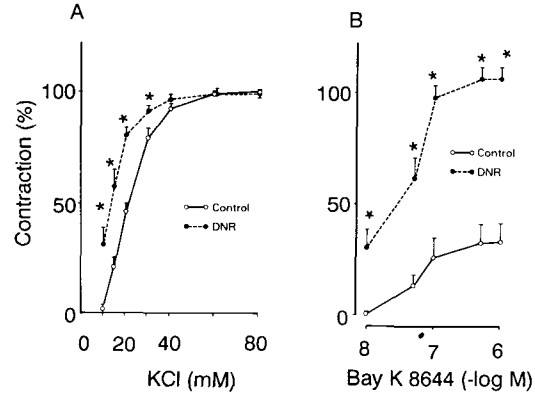

FIG. 2. Effect of pretreatment of aortic strips with 17·7 μM daunorubicin (DNR) for 60 min upon the concentration-response relationships for KCl(A)- and BAY K 8644(B)-induced contractions in rat aortae. Responses are expressed in terms of the percentage of contraction induced by 80 mM KCl in the control pretreated with 0·9% saline. Asterisks denote significant differences from the control ($P < 0.05$) (n = 6).

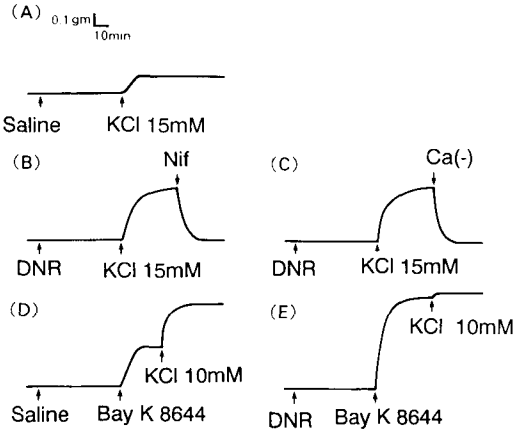

FIG. 1. Representative tension recording of the contractile responses of aorta to KCl (15 mM) and BAY K 8644 (1 μM) under various conditions. DNR: daunorubicin (17·7 μM), Nif: nifedipine (1 μM), Ca(−): in calcium-free solution.

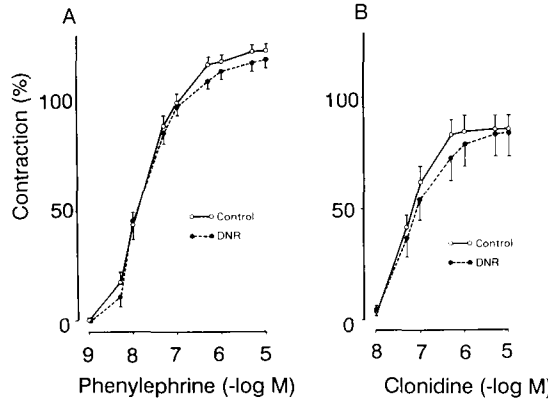

FIG. 3. Effect of pretreatment of aortic strips with 17·7 μM daunorubicin (DNR) for 60 min upon the concentration-response relationships for phenylephrine (A)- and clonidine (B)-induced contractions in rat aortae. Responses are expressed in terms of the percentage of contraction induced by 80 mM KCl in the control pretreated with saline (n = 6).

Discussion

Our data show that daunorubicin potentiates the vasocontractile responses to KCl and BAY K 8644. Both KCl and BAY K 8644 induce vasocontraction by activation of the voltage-dependent calcium channel and the subsequent influx of extracellular calcium (Godfraind & Kaba 1972; Schramm et al 1983a, b). We also studied the effect of daunorubicin upon the contractile responses to phenylephrine and clonidine, which are mediated by activation of vascular α_1- and α_2-adrenoceptors, respectively. Since the vasocontractile response to α-adrenoceptor agonists are known to be modulated by the endothelium (Eglème et al 1984; Lues & Schümann 1984), the endothelium was removed by gentle rubbing of the intimal surface of the aortic strips to eliminate any effects of endothelium-derived relaxing factors. Phenylephrine is known to activate vascular α_1-adrenoceptors, which leads to breakdown of membrane phosphoinositides and then to the induction of a contraction which is dependent upon extra- and intracellular calcium (Chiu et al 1986, 1987). Clonidine is generally known to activate vascular α_2-adrenoceptors, which leads to opening of the receptor-operated calcium channel and then to the induction of a contraction which is dependent upon extracellular calcium (Godfraind & Miller 1982). However, the presence of α_2-adrenoceptors in rat aorta is controversial. Some studies indicate that responses of rat aorta to α_2-selective agonists are mediated by α_1-adrenoceptors from the experiments using selective α_1- and α_2-adrenoceptor agonists and antagonists (Digges & Summers 1983; Macia et al 1984). These findings and our result that daunorubicin potentiated the contractions caused by KCl and BAY K 8644, but not those by phenylephrine and clonidine, suggest that daunorubicin specifically potentiates the contraction mediated by activation of the voltage-dependent calcium channel.

Recently, Wanstall & O'Donnell (1989) demonstrated that contractile responses mediated by the voltage-dependent calcium channel are lower in aortae from aged rats compared with young rats and postulated that the resting membrane potential may be more negative in the aortae from the aged rats. To elucidate the facilitative effect of daunorubicin upon activation of the voltage-dependent calcium channel, we used rats 6–8 months old. In young rats (8–9 weeks old), the facilitative action of daunorubicin on KCl- and BAY K 8644-induced contractions was significant but less than that in the elderly rats (data not shown).

Although the effects of anthracycline antibiotics on vascular smooth muscle cell membrane are not known, there are several reports describing those on skeletal and cardiac muscle cell membranes in relation to Ca^{2+} movement. Banning et al (1987) showed that daunorubicin inhibits the cardiac contractile force by reduction of the dihydropyridine-sensitive binding sites in the cardiac sarcolemma. On the other hand, Abramson et al (1988) reported that in skeletal muscle, daunorubicin and doxorubicin interact with the ryanodine receptor complex, stimulate Ca^{2+} release and trigger transient contractions of muscle fibres. In the present study, we found that the maximal contraction by KCl was not changed by pretreatment with daunorubicin, while that by BAY K 8644 was markedly potentiated. As the maximal contractile response to KCl was obtained at 80 mM and was lower than that to phenylephrine or noradrenaline (phenylephrine, noradrenaline maximum; $122{\cdot}2 \pm 2{\cdot}9$, $130{\cdot}7 \pm 2{\cdot}6\%$ of 80 mM KCl contraction, respectively), the maximum response to KCl was not the tissue maximum. Furthermore, the maximal contractile response to KCl was increased by pretreatment with a low concentration of BAY K 8644 (Mikkelsen et al 1985). Therefore, the fact that the maximum to KCl was not potentiated by daunorubicin, whereas that to BAY K 8644 was, does not simply reflect that the K^+ maximum, unlike the BAY K 8644 maximum, was already close to the tissue maximum. KCl activates the voltage-dependent calcium channel via membrane depolarization, while BAY K 8644 activates it directly (Bolton 1979; Schramm et al 1983b). A previous report stated that maximal contractile respone to BAY K 8644 was remarkably increased in vessels partially depolarized by KCl from the experiments using rat aortae (Wanstall & O'Donnell 1989). As shown in Fig. 1D, E, the maximal contractile response to BAY K 8644 was greatly increased by partial depolarization with KCl (10 mM, final concentration) in the control solution but only slightly increased by the partial depolarization in the solution with daunorubicin. Therefore, it is postulated that the potentiating effect of daunorubicin on the contraction mediated by activation of the voltage-dependent calcium channel may be due to the same mechanism as that of KCl contraction, namely membrane depolarization. In the previous studies using rabbit mesenteric and femoral arteries, the contractile responses to noradrenaline (both an α_1- and an α_2-adrenergic agonist) was found to be increased by membrane depolarization (Abel et al 1981; Nelson et al 1988). In this study using rat aorta, however, neither sensitivity nor reactivity of noradrenaline contraction was significantly different between the control K^+ (4·7 mM) and the high K^+ (10 mM) solution [EC50: $2{\cdot}31$ ($+0{\cdot}65$ to $-0{\cdot}56$) $\times 10^{-8}$ M, $1{\cdot}78$ ($+0{\cdot}62$ to $-0{\cdot}46$) $\times 10^{-8}$ M; maximal contractile force: $130{\cdot}7 \pm 2{\cdot}6\%$, $130{\cdot}6 \pm 2{\cdot}7\%$, respectively]. Therefore, it seems that the effect of membrane depolarization upon the α-adrenergic contractile response varies with the animal species or the vessel loci. Our recent report has shown that aclarubicin, another anthracycline antibiotic, attenuates the aortic contractile responses to KCl and phenylephrine in in-vitro and ex-vivo experiments (Wakabayashi et al 1989a). Thus, it is likely that the vascular action of daunorubicin is opposite to that of aclarubicin.

In conclusion, daunorubicin at a concentration which does not induce contraction potentiates the contractile responses mediated by activation of the voltage-dependent calcium channel in rat aorta.

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