Nishihata, T., Rytting, J. H., Caldwell, L., Yoshioka, S., Higuchi, T. (1982) Adjuvant effects on rectal absorption. In: Bundgard, H. Hansen, A. B. and Kofard H. (eds) Optimization of drug delivery, Alfred Benzone Symposium 17, Munksgard, Copenhagen, pp. 17–35

Nishihata, T., Rytting, J. H., Kamada, A., Higuchi, T., Routh, M., and Caldwell, L. (1983) Enhancement of rectal absorption of insulin using salicylate in dogs. J. Pharm. Pharmacol. 35: 148–151

Nishihata, T., Okamura, Y., Kamada, A., Higuchi, T., Yagi, T., Kawamori, R. and Shichiri, M. (1985) Enhanced bioavailability of insulin after rectal administration with enamine as adjuvant in depancreatized dogs. Ibid. 37: 22–26

Nishihata, T., Okamura, Y., Inagaki, H., Sudoh, M., Kamada, A., Yagi, T., Kawamori, R., Schichiri, M. (1986a) Trial of rectal insulin suppositories in healthy humans. Int. J. Pharm. 34: 157–161

Nishihata, T., Sudoh, M., Yamazaki, M., Kamada, A. (1986b) Inhibition of gluconeogenesis by salicylate and 5-methoxysalicylate in liver of normal and alloxan-diabetic rats. Chem. Pharm. Bull. 33: 5086–5092

Nishihata, T., Sudoh, M., Inagaki, H., Kamada, A., Yagi, T., Kawamori, R., Shichiri, M. (1987) An effective formulation for an insulin suppository; examination in normal dogs. Int. J. Pharm. 38: 83–90

Ritschel, W. A., Ritschel, G. B. (1984) Rectal administration of insulin. In: Glas B. & De Blay J. (eds.) Rectal therapy, Prous, Barcelona, pp. 67–83

Sithigorngul, P., Burton, P., Nishihata, T., Caldwell, L. (1983) Effects of sodium salicylate on epithelial cells of the rectal mucosa of the rat: a light electron microscopic study. Life Sci. 33: 1025–1032

Woods, H. F., Stubbs, W. A., Johnson, G. (1974) Inhibition by salicylate of gluconeogenesis in the isolated perfused rat liver. Clin. Exp. Pharmacol. Physiol. 1: 335–340

Yue, D. K., Mclenna, S., Handeksman, P. J., Delbridge, L., Reeve, T., Turtle, J. R. (1984) The effect of salicylate on nonenzymatic glucosylation and thermal stability of collagen in diabetic rats. Diabetes 33: 745–751

Yue, D. K., Mclenna, S., Handelsma, P. J., Delbridge, L., Reeve, T., Turtle, J. R. (1985) The effect of cyclo-oxygenase and lipo-oxygenase inhibitors on the collagen abnormalities of diabetic rats. Ibid. 34: 74–78

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Vasocontractile action of daunorubicin

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Abstract—Daunorubicin (35·5–142 μmol L^{-1}) induced in rat aortic strips a contraction with slow onset and a gradual development of tension on which neither phentolamine nor bromophenacyl bromide pretreatment had an effect. The contraction was not altered by removal of the endothelium but it was suppressed in calcium-free solution or by preincubation with nifedipine. These results suggest that daunorubicin directly stimulates vascular smooth muscle and induces a contractile response which is mainly dependent upon extracellular calcium.

Daunorubicin (daunomycin) is a potent antineoplastic anthracycline derivative, but its cytotoxicity to cardiac muscle limits its use in antineoplastic therapy (Tan et al 1967). It generally displays chronic depressive action on cardiac muscle, but in some species, it has an acute positive inotropic effect (Gibbs 1985), the precise mechanism of which, like its effect on the vascular system, remains unknown. We have investigated the acute effect of the drug on rat aortic strips and found it to have a vasocontractile action.

Materials and methods

Contraction study. Thoracic aortas were excised from male Wistar rats (400–450 g) and placed in Krebs-Ringer bicarbonate solution (mM: NaCl 118, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25 and glucose 10). After removal of the connective tissue, helical strips (2 mm wide × 15 mm long) were prepared and suspended vertically in 10 mL organ chambers filled with the above solution (37°C, pH 7·4) through which 95% O_2–5% CO_2 was bubbled. After 1 h of equilibrium with a resting tension of 1 g, changes in isometric force were recorded. In some preparations, the endothelium was removed by gentle abrasion of the intimal surface with sandpaper. Denudation of the endothelium was confirmed functionally by the disappearance of the 10^{-5} M acetylcholine-induced relaxing response of the 10^{-7} M noradrenaline-precontracted vessel (Wakabayashi et al 1987). Contractile responses to daunorubicin were expressed in terms of the percentage of contraction by 60 mM KCl in each strip. The concentration of each drug was expressed as the final concentration in the organ bath.

Substances. Nifedipine (Sigma) was dissolved in ethanol to give a stock solution of 10^{-3} M. The final concentration of ethanol in 10^{-6} M nifedipine was 0·1%, which did not affect the contractile response to daunorubicin. All experiments with nifedipine were conducted in the dark. Daunorubicin (Daunomycin, Meiji Seika) and phentolamine (Ciba-Geigy) were dissolved in 0·9% saline. Bromophenacyl bromide (Wako) was dissolved in ethanol. The concentration of ethanol in 3×10^{-6} M bromophenacyl bromide was 0·3%, which did not affect the contraction by daunorubicin.

Statistical analysis. All the values represent mean ± s.e.m. The data were analysed by Student's t-test and $P < 0.05$ was defined as significant.

Results

Daunorubicin induced a contractile response of the rat aorta which showed a slow onset and a gradual increase in tension. About 1 h was required for this contraction to reach a plateau. Incubation of the aortic strip with 0·9% saline, a solvent of daunorubicin, for 60 min did not affect the basal vascular tone (Fig. 1A). The vasocontractile action of daunorubicin was initiated at a concentration of 35·5 μM and the maximal contraction was attained at 142 μM (Fig. 1B). Figure 1C shows the effects of several inhibitors and conditions on the daunorubicin-induced contractile response of the rat aorta. This response was suppressed by pretreatment of the aorta with nifedipine and also in calcium-free solution. Pretreatment with either phentolamine or bromophenacyl bromide did not affect the contractile

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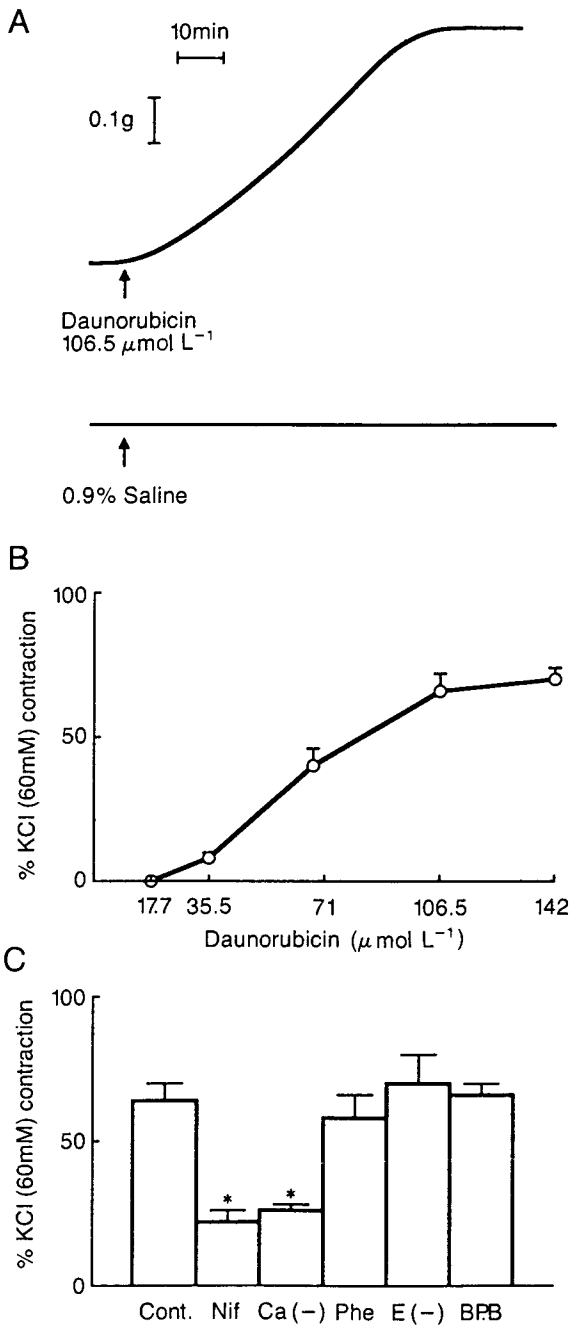

FIG. 1. A. Typical contractile response curve by daunorubicin (106·5 μM) in rat aortas. B. Dose-response relationship of contractile response to daunorubicin added cumulatively to the organ chambers. Data are expressed as mean ± s.e.m. (n = 5). C. Effect of several conditions and inhibitors upon daunorubicin-induced contractile response. Experiments in calcium-free solution (Ca(−)) were performed as follows: the vessels were rinsed quickly 3 times with calcium-free Krebs-Ringer solution containing 0·1 mM ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*'-tetraacetic acid (EGTA) and allowed to equilibrate in this solution for an additional 15 min. The vessels were then stimulated with 106·5 μM daunorubicin. E(−) indicates experiments using vessels without the endothelium. Experiments using inhibitors were performed as follows: the vessels were pretreated with each inhibitor [10^{-6}M nifedipine for 60 min (Nif), 2×10^{-6}M phentolamine for 60 min (Phe.) or 3×10^{-6}M bromophenacyl bromide for 60 min (BPB)] and contraction was induced by daunorubicin (106·5 μM). Data are expressed as mean ± s.e.m. (n = 5–7) and asterisks indicate statistically significant differences ($P < 0.05$) from the controls (Cont).

response to daunorubicin. The response to daunorubicin was not altered by removal of endothelium. Neither phenotolamine nor bromophenacyl bromide pretreatment altered the dose-response relationship for daunorubicin nor did removal of the endothelium (data not shown).

Discussion

Daunorubicin induced a gradual contraction in rat aorta which was not affected by pretreatment with phentolamine, an α-adrenergic receptor antagonist, or removal of the endothelium. Therefore, neither endogenous noradrenaline nor endothelial metabolites are involved in the contractile response to daunorubicin. Also, this response was not mediated by α-adrenergic receptor stimulation of vascular smooth muscle. Bromophenacyl bromide, a known phospholipase A_2 inhibitor, did not affect the contraction induced by daunorubicin. This means that metabolites of arachidonic acid from aorta are not involved in the contraction. Daunorubicin-induced vasoconstriction was inhibited by pretreatment of the aorta with nifedipine, a calcium channel blocker, and also in calcium-free solution. This implies that calcium influx across the calcium channel is mainly involved in this contraction. About 30% of the daunorubicin-induced contraction remained in tissue in calcium-free solution and after pretreatment with nifedipine. Thus, calcium from intracellular storage is partially involved in this contraction.

The peak blood concentration of daunorubicin during anti-leukaemic chemotherapy was reported to be about 8 μM (Burns & Dow 1980). The concentration of daunorubicin for inducing the vasocontractile action in-vitro is about 4·5 times its peak blood concentration under chemotherapy.

References

Burns, J. H., Dow, J. W. (1980) Daunorubicin-induced myocardial failure: reversal by digoxin of an inability to use ATP for contraction. J. Mol. Cell Cardiol. 12: 95–108

Gibbs, C. L. (1985) Acute energetic effects of daunomycin on rabbit heart muscle. J. Cardiovasc. Pharmacol. 7: 556–561

Tan, C., Tasaka, H., Yu, K. P., Murphy, M. L., Karnofsky, D. A. (1967) Daunomycin, an antitumor antibiotic, in the treatment of neoplastic disease. Cancer 20: 333–353

Wakabayashi, I., Hatake, K., Kimura, N., Kakishita, E., Nagai, K. (1987) Modulation of vascular tonus by the endothelium in experimental diabetes. Life Sci. 40: 643–648