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Inhibition by diethylcarbamazine of acetylcholine-induced endothelium-dependent relaxation of rabbit aorta: are leukotrienes involved?

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Acetylcholine caused relaxations of preconstricted rabbit aortic strips if the endothelium was intact. These relaxations were reversed in a concentration-dependent manner by diethylcarbamazine, an inhibitor of slow-reacting substance and leukotriene formation. However, when exogenous leukotrienes (LTA_4, LTB_4, LTC_4 and LTD_4) were added to the precontracted arterial strips none of them caused relaxation, showing that they are unlikely to be involved as mediators in the acetylcholine-induced relaxation of rabbit aorta.

Acetylcholine (ACh) causes relaxations of isolated blood vessels by a mechanism dependent upon intact endothelial cells (Furchgott & Zawadzki 1980; Chand & Altura 1981). This effect of ACh is resistant to cyclooxygenase inhibition but sensitive to inhibitors of phospholipase A_2 and lipoxygenases (Furchgott & Zawadzki 1980; Chand & Altura 1981; Singer & Peach 1983; Förstermann & Neufang 1984). It has therefore been postulated that lipoxygenase products (hydroperoxy/hydroxy fatty acids or leukotrienes) might be mediators of the endothelium-dependent relaxation (Furchgott & Zawadzki 1980; Chand & Altura 1981).

Diethylcarbamazine has been shown to inhibit the synthesis of slow-reacting-substance or cysteinyl-leukotriene-like material in several systems (Orange et al 1971; Engineer et al 1978; Piper & Temple 1981; Mathews & Murphy 1982). We therefore tested the effect of this drug on the ACh-induced endothelium-dependent relaxation of rabbit aortic strips.

Methods

Helically cut strips of rabbit thoracic aorta (15 × 2 mm, about 30 mg wet wt) were set up in organ baths (3·5 ml) as previously described (Förstermann et al 1984), care being taken to avoid damage of the intimal surface. In some experiments the endothelium was removed by careful abrasion with a razor blade. The bath medium (Krebs-bicarbonate solution of the following composition (mM): NaCl 120·0, KCl 4·75, $NaHCO_3$ 25·0, KH_2PO_4 1·2, $MgSO_4$ 1·2, CaCl 1·7 and glucose 6·4) was changed every 12 min throughout. Changes in tissue length were recorded with an isotonic lever system. Drugs were added to the organ bath in a volume of 50 µl. Contractions of the strips were elicited at 1 h intervals with 10^{-7} M noradrenaline (NA). Seven min after NA, when a stable contraction plateau had been reached, different concentrations of ACh were added to the organ bath to produce relaxations. Different concentrations of diethylcarbamazine (Sigma, Munich, FRG) were always added to the bath medium 36 min before the next contraction–relaxation period. When the effect of leukotrienes (LTs) was tested, they were added to the organ bath instead of ACh (7 min after NA). LTA_4 was obtained as the methylester (Paesel, Frankfurt, FRG) and hydrolysed to the sodium salt with 1 mM NaOH pH 11·0. Since LTA_4 is unstable at neutral pH, the pH of the solution was readjusted to 7·4 5 s before adding it to the organ bath. In some experiments the stable LTA_4-methylester was given directly to the aortic tissue. LTB_4 (Paesel, Frankfurt, FRG) and LTC_4 and LTD_4 (both generous gifts of Dr J. Rokach, Merck-Frosst, Pointe-Claire - Dorval, Canada) were obtained and tested as free acids. All concentrations of drugs given refer to the free bases or acids respectively.

Results and discussion

ACh induced concentration-dependent relaxations of rabbit aortic strips that had been contracted by NA. The maximal effect was reached with 10^{-6} M (relaxation of

* Correspondence.

the strips to 55 ± 6%, mean ± s.e.m., n = 7, of the precontraction level), and 10^{-7} M ACh induced about a half-maximal relaxation. De-endothelialized strips slightly contracted in response to ACh.

As reported by several authors, the relaxing effect of ACh can be reversed by different inhibitors of lipoxygenase (Furchgott & Zawadzki 1980; Chand & Altura 1981; Singer & Peach 1983; Förstermann & Neufang 1984). Also diethylcarbamazine concentration-dependently inhibited the relaxation induced by ACh (10^{-7} M) as shown in Fig. 1. Diethylcarbamazine has previously been shown to inhibit the formation of slow-reacting substance of cysteinyl-leukotrienes in several cells and tissues (Orange et al 1971; Engineer et al 1978; Piper & Temple 1981; Mathews & Murphy 1982). A recent report suggested that these effects might not be due to an interference of the drug with C-5-lipoxygenase but with LTA_4-synthetase (Mathews & Murphy 1982). Thus leukotrienes formed subsequent to the action of LTA_4-synthetase might be mediators of the ACh-induced relaxation.

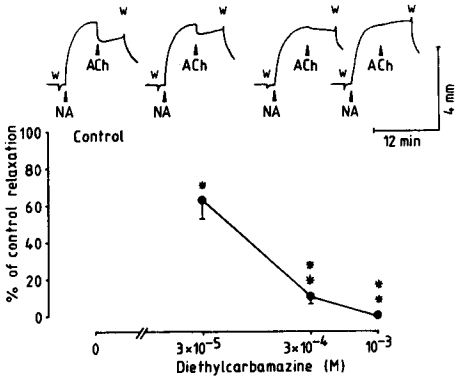

FIG. 1. Effect of different concentrations of diethylcarbamazine on the acetylcholine (ACh, 10^{-7} M)-induced relaxation of rabbit aortic strips precontracted with noradrenaline (NA, 10^{-7} M). The upper panel shows an original recording from one of the strips in normal bath medium (control) and upon exposure to the three different concentrations of diethylcarbamazine indicated on the abscissa (bottom panel) (W: wash-out, change of bath medium). The lower panel shows mean results ± s.e.m., n = 4–6. Relaxations to 10^{-7} M ACh in normal bath medium are taken as 100%. Asterisks indicate statistically significant inhibitions of these relaxations, $*P < 0.01$, $**P < 0.001$ (paired values test).

We therefore investigated the effect of such leukotrienes on the precontracted rabbit aorta. LTA_4 (10^{-7} and 10^{-6} M) or LTA_4-methylester (10^{-6} and 10^{-5} M) were either ineffective or produced small additional contractions (5% of the effect of 10^{-7} M NA) of the aortic strips. The same held true for LTC_4 (10^{-7} and 10^{-6} M) and LTD_4 (10^{-7} and 10^{-6} M). LTB_4 (10^{-7} and 10^{-6} M) consistently had no effect at all. These data indicate that LTA_4, LTB_4, LTC_4 and LTD_4 are unlikely to mediate the relexant effect of ACh on aortic strips.

If diethylcarbamazine was a specific inhibitor of LTA_4-synthetase as suggested by Mathews & Murphy (1982), another, possibly unknown derivative of LTA_4 has to be postulated as the factor mediating relaxation. However, evidence for an inhibition of LTA_4-synthetase by diethylcarbamazine has only been presented by the above authors for concentrations as low as 5×10^{-6} M, whereas higher concentrations ($\geq 3 \times 10^{-5}$ M) are necessary to significantly inhibit the ACh-relaxation (Fig. 1). Similarly much higher concentrations of diethylcarbamazine were required to obtain inhibition of the immunological release of slow-reacting substance from human and guinea-pig lung (Orange et al 1971, Engineer et al 1978; Piper & Temple 1981). Therefore it cannot be excluded that the high concentrations of diethylcarbamazine used here, and in other studies also inhibit lipoxygenase, and the mechanism of action of diethylcarbamazine in reversing the ACh-induced relaxation might be similar to that of lipoxygenase inhibitors (Förstermann & Neufang 1984).

In conclusion, our data demonstrate that diethylcarbamazine inhibits the ACh-induced endothelium-mediated relaxation in a concentration-dependent manner probably by acting on lipoxygenase. The leukotrienes LTA_4, LTB_4, LTC_4 and LTD_4 are unlikely to be mediators of this relaxation.

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