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The effect of lipoxygenase inhibitors and diethylcarbamazine on the immunological release of slow reacting substance of anaphylaxis (SRS-A) from guinea-pig chopped lung

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When challenged with antigen, chopped lung tissue from sensitized guinea-pigs releases histamine, SRS-A and prostaglandins (PGs) (Morris et al 1980). In the presence of indomethacin, which inhibits the cyclo-oxygenase pathway for the conversion of arachidonic acid to prostaglandins, this immunological release of SRS-A was enhanced (Engineer et al 1978). The addition of exogenous arachidonic acid further increased SRS-A release, indicating that the formation of SRS-A in guinea-pig lung involves the arachidonate lipoxygenase pathway (Burka & Paterson 1980; Morris et al 1980). These observations suggest that drugs which are lipoxygenase inhibitors may inhibit the immunological release of SRS-A.

Higgs et al (1979) reported that BW 755C {3-amino-1-[m-(trifluoromethyl)-phenyl]-2-pyrazoline} a structural analogue of the anti-anaphylactic compound 1-phenyl-3-pyrazolidone (Blackwell & Flower 1978), inhibited arachidonate metabolism by both the cyclo-oxygenase and lipoxygenase pathways in horse blood platelets in vitro.

We have determined the effects of BW 755C and of its *p*-chloroanalogue CLI (Adcock et al 1978) on the immunological release of SRS-A, histamine and $PGF_{2\alpha}$ from guinea-pig chopped lung. The effects of diethylcarbamazine in the guinea-pig chopped lung model have also been measured. Diethylcarbamazine has previously been shown to inhibit SRS-A release from human and monkey lung fragments (Ishizaka et al 1971; Orange et al 1971) and from guinea-pig isolated perfused lung (Engineer et al 1978).

Lungs from male guinea-pigs (Dunkin-Hartley, 300–350 g) previously sensitized to ovalbumin (Sigma Grade II, 100 mg, i.p. and s.c.) were perfused free of blood with Tyrode's solution at 37 °C and then chopped into pieces (1 mm^3) with a McIlwain tissue chopper. After washing, the lung fragments were incubated in Tyrode's solution at 37°C as previously described (Morris et al 1980). In each experiment all test and control samples were taken from the same pair of lungs and 4 or 5 such experiments were performed for each concentration of BW755C and diethylcarbamazine. The lung fragments were pre-incubated for 15 minutes before challenge with ovalbumin (Sigma Grade III, 1 $\mu g\ ml^{-1}$) and incubation continued for a further 15 min. The supernatant was assayed for SRS-A (bioassay) histamine (fluorimetric assay) and $PGF_{2\alpha}$ (radioimmuno-assay) as previously described (Morris et al 1980). The effect of each drug concentration on the release of SRS-A, histamine and $PGF_{2\alpha}$ was expressed as a percentage of the control value obtained in the absence of drug.

Each of the lipoxygenase inhibitors caused concentration-related reductions in SRS-A release, at concentrations from $1{\cdot}1 \times 10^{-4}$ mol $litre^{-1}$ for BW755C and from 4×10^{-5} to 4×10^{-4} mol $litre^{-1}$ for CLI (Fig. 1). Indomethacin, which blocks the cyclo-oxygenase pathway, slightly increased antigen-induced SRS-A release in the absence of added drugs and enhanced their inhibitory effect on SRS-A release as follows: BW755C, at concentrations of 11, 38 and 110 μmol $litre^{-1}$, in the absence of indomethacin caused percentage reductions of SRS-A release of $6{\cdot}8 \pm 8{\cdot}8$, $20{\cdot}3 \pm 1{\cdot}8$ and $52{\cdot}8 \pm 5{\cdot}8$ (mean values ± s.e.m. from 5 experiments), while in the presence of indomethacin the corresponding percentage values were $19{\cdot}7 \pm 7{\cdot}1$, $44{\cdot}3 \pm 1{\cdot}3$ and $64{\cdot}5 \pm 3{\cdot}2$ (3 experiments). Histamine release was unaffected by the lower concentration range of BW755C and CLI, but highest drug concentrations above 10^{-4} mol $litre^{-1}$ caused a significant reduction in histamine release. Neither drug caused mediator release in the absence of antigen.

These results indicate that immunological release of SRS-A requires an intact lipoxygenase pathway as proposed by Morris et al (1980). Nijkamp & Ramakers (1980) found that antigen-induced bronchoconstriction in guinea-pig isolated lungs was inhibited by BW755C and antagonized by the SRS-A antagonist FPL 55712 {sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxy]-4-oxo-8-propyl-4^{14}-chromene-2-carboxylate} (Augstein et al 1973), which supports the causal role of SRS-A and the involvement of the lipoxygenase pathway in anaphylactic bronchoconstriction guinea-pigs.

$PGF_{2\alpha}$ release was inhibited dose-dependently by BW755C(2 experiments). The IC50 was $3{\cdot}8 \times 10^{-6}$ mol $litre^{-1}$ for $PGF_{2\alpha}$ release compared with 1×10^{-4} mol $litre^{-1}$ for SRS-A release. This was predictable from the report of Higgs et al (1979) that BW755C was a more potent inhibitor of arachidonate cyclo-oxygenase than of lipoxygenase in platelets. CLI also inhibited $PGF_{2\alpha}$ release.

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Diethylcarbamazine citrate caused a significant dose-related inhibition of SRS-A release at concentrations from 10^{-4} to 5×10^{-3} mol litre^{-1}. The presence of indomethacin had little effect on this degree of inhibition. Histamine release was unaffected by all but the highest concentration of diethylcarbamazine (5×10^{-3} mol litre^{-1}) which caused a significant reduction. In this respect, these results for diethylcarbamazine resemble those of the lipoxygenase inhibitors in demonstrating a selective inhibition of SRS-A release at concentrations that do not significantly inhibit histamine release. Orange et al (1971) reported that diethylcarbamazine inhibited the immunological release of both SRS-A and histamine from human chopped lung, IC50 10^{-3} mol litre^{-1}. Ishizaka et al (1971) obtained similar results with monkey lung, IC50 4×10^{-3} mol litre^{-1}, and showed

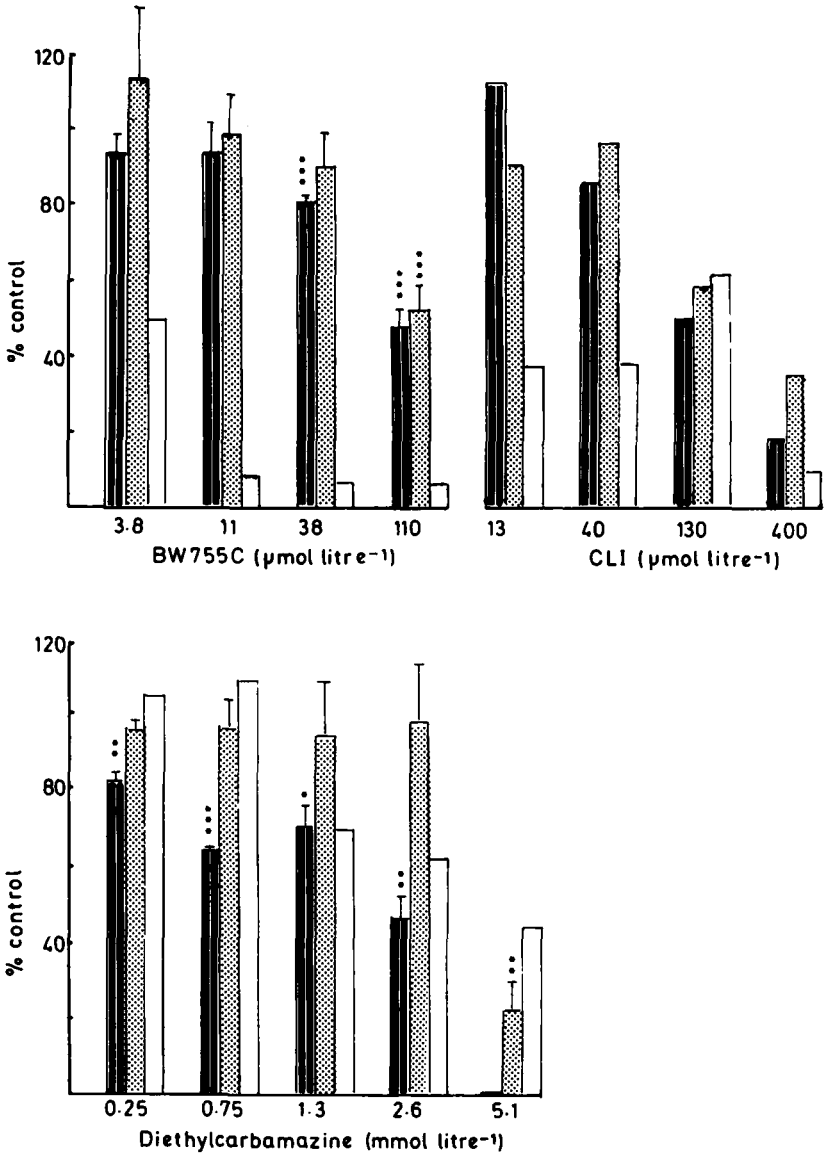

FIG. 1. Effect of BW755C, CLI and diethylcarbamazine on the release of SRS-A, histamine and prostaglandin $F_{2\alpha}$ from sensitized guinea-pig chopped lung challenged with antigen. Striped columns: SRS-A, stippled columns: histamine, open columns: $PGF_{2\alpha}$. Values are expressed as percentages of drug-free control release in each experiment, and are mean values, with vertical bars representing s.e. means. Each column for SRS-A and histamine is the mean of 4–5 experiments for BW755C and diethylcarbamazine, and of 2 experiments for CLI and all $PGF_{2\alpha}$ assays. Significance (paired t-test): * $P<0{\cdot}02$, ** $P<0{\cdot}01$, *** $P<0{\cdot}001$.

synergism between the actions of diethylcarbamazine and isoprenaline similar to that between methylxanthines and isoprenaline, indicating that an elevation of cyclic(c) AMP might be the mechanism of the inhibition of mediator release. Engineer et al (1978) with guinea-pig isolated perfused lung showed inhibition of both SRS-A and histamine release by $2{\cdot}6 \times 10^{-3}$ mol litre^{-1}. Our results show a greater sensitivity of guinea-pig chopped lung to diethylcarbamazine, since significant inhibition of SRS-A release is seen at concentrations of diethylcarbamazine of $2{\cdot}5 \times 10^{-4}$ and $7{\cdot}5 \times 10^{-4}$ mol litre^{-1}. Engineer et al (1978) also found a dose-dependent increase in PGE release by 5×10^{-4} and $2{\cdot}5 \times 10^{-3}$ mol litre^{-1} of diethylcarbamazine, and proposed that prostaglandins and related compounds released in anaphylaxis may influence the release of SRS-A, perhaps by way of an interaction with cyclic nucleotide. $PGF_{2\alpha}$ release from guinea-pig chopped lung (2 experiments here) was unchanged by the lower concentrations ($2{\cdot}5$ and $7{\cdot}5 \times 10^{-4}$ mol litre^{-1}) of diethylcarbamazine, and reduced by the higher concentrations ($1{\cdot}3$, $2{\cdot}6$ and $5{\cdot}1 \times 10^{-3}$ mol litre^{-1}). Diethylcarbamazine ($1{\cdot}3 \times 10^{-3}$ mol litre) in the absence of antigen increased $PGF_{2\alpha}$ release by 26%. $PGF_{2\alpha}$ release appears here to be related to SRS-A release, supporting the evidence of Engineer et al (1977) that SRS-A released in anaphylaxis may cause the release of prostaglandin-like substances.

Whereas the inhibition of immunological release of SRS-A and $PGF_{2\alpha}$ by BW755C and CLI can be explained by their inhibition of lipoxygenase and cyclooxygenase, the inhibitor action of diethylcarbamazine appears to involve a different mechanism that may depend on cAMP.

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Preliminary observations on the excretion of acebutolol and its acetyl metabolite in the urine and faeces of man

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Less than half an oral dose of acebutolol hydrochloride is recovered in human urine (Kaye et al 1976; Meffin et al 1976). It has been suggested that the remainder is unabsorbed and appears in faeces. However, acebutolol and its major metabolite, diacetolol—an acetyl analogue are excreted in human bile (Kaye & Oh 1976) and acebutolol can cross the gut wall from the systemic circulation (Collins & George 1976; George & Gruchy 1979).

We have assessed urinary and faecal excretion of the drug and its metabolites in a healthy male volunteer. Urine and faeces were collected after the administration of one tablet containing 400 mg of acebutolol. Several weeks later urine and faeces were collected after a single intravenous dose of 100 mg of acebutolol. Collections were made on each occasion for the 24 h preceding the dose and daily for 4 days after the dose.

Urine collected after the oral administration of acebutolol contains acebutolol and diacetolol, and small amounts of a second metabolite, M & B 17, 127—the free amine derivative of acebutolol (Andresen & Davis 1979). The assay used is specifically capable of quantitating acebutolol and both metabolites. The procedure for faeces was as follows: 1 g of homogenized faeces was weighed in a centrifuge tube to which was added 2 ml of water and a suitable amount of an internal standard M & B 17, 764—the propionamido analogue of acebutolol; after mixing, the compounds of interest were extracted into ethyl acetate under alkaline conditions, back extracted into acid, and then extracted into chloroform after the addition of excess alkali. For urine, the initial extraction and back extraction were omitted. Each chloroform extract of faeces and urine was evaporated to dryness and the residue taken up in 100 μl of the mobile phase (see below). The compounds of interest were separated and quantitated by reversed-phase high performance liquid chromatography using an analytical column of Spherisorb S5 ODS, protected by a small guard column of Whatman Co:Pell ODS, a mobile phase of water–methanol–triethylamine

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