MDRG. A role for calcium antagonists in reversing drug resistance deserves extensive exploration.

In summary, a photoactive calcium antagonist, [^{3}H]azidopine, can specifically radiolabel the MDRG. Other calcium antagonists and drugs used to select our resistant cells influenced [^{3}H]azidopine labeling of the MDRG. Analysis of tryptic peptides from the [^{3}H]azidopine-labeled MDRG suggested that this calcium antagonist has a specific binding site on the MDRG.

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Ascorbic acid-induced binding of [^{125}I]-iodocyanopindolol to non-beta-adrenoceptor sites in guinea-pig trachea

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[^{125}I]-Iodocyanopindolol (I-CYP) is a potent, selective radioligand for beta-adrenoceptors [1–4]. However, recent evidence indicates that I-CYP also binds with high affinity to a population of non-beta-adrenoceptor sites in dog kidney [5]. Furthermore, we have recently shown that in the presence of ascorbic acid, I-CYP binds to non-beta-adrenoceptor sites in guinea-pig trachea [6]. The present study further examines the characteristics of ascorbic acid-induced I-CYP binding in airway tissue.

Methods

Tissue preparation, I-CYP binding and autoradiography. Male guinea-pigs (SR/C Tricolour; 400–450 g) and male Wistar rats (220–250 g) were stunned and exsanguinated and the trachea removed. Bronchi (2–3 mm i.d.) were also dissected from lung from pigs freshly slaughtered at a local abattoir. Airway tube segments were submerged in 6% dextran 70, containing 5% dextrose (Macrodex, Pharmacia) and rapidly frozen in isopentane cooled in liquid nitrogen. Each frozen block contained airway tissue from at least 3 separate animals. Serial, transverse, 10-μm sections were cut at −30° and mounted and thawed onto washed gelatinized glass slides. Binding and autoradiographic experiments using I-CYP (5–320 pM; Amersham), were conducted as previously described [6]. In some experiments, tissue sections were exposed to I-CYP (50 pM) for 2 hr in the presence of ascorbic acid, dithiothreitol, L-cysteine (Sigma Chemical Co.) or sodium metabisulphite (BDH) (1 nM–1 mM). Unless otherwise stated, propranolol (10 μM) (ICI) was routinely included to abolish I-CYP binding to beta-adrenoceptors. Various cations as well as drugs from several pharmacological groups were also tested for their effects on I-CYP binding to non-beta-adrenoceptor sites in the presence of ascorbic acid.

Results and discussion

Specific I-CYP binding in guinea-pig and rat trachea and in pig bronchus was saturable, involving high affinity sites (dissociation constant K_d = 69 ± 9; 80 ± 14 and 66 ± 12 pM respectively). Specific binding was reduced to background levels in the presence of propranolol (10 μM) or (−)-isoprenaline (200 μM), indicating reversible binding was to

beta-adrenoceptors. In guinea-pig trachea exposed to propranolol (10 μM), ascorbic acid caused a time-dependent increase in I-CYP (50 pM) binding. Binding reached equilibrium after approximately 120 minutes. This incubation time was used in all subsequent experiments. Ascorbic acid had no such effect in rat trachea or pig bronchus. It is interesting to note that unlike the rat, which can synthesize ascorbic acid, the guinea-pig is dependent upon a dietary supply [7].

Ascorbic acid caused a maximal increase in I-CYP binding to non-beta-adrenoceptor sites in guinea-pig trachea at a concentration of 10 μM (Fig. 1). This was 11 ± 1 times greater than that associated with binding to beta-adrenoceptors. Dithiothreitol (DTT) and L-cysteine also caused concentration-dependent increases in I-CYP binding to non-beta-adrenoceptor sites, which were maximal at 10 μM. However, these were approximately 53 and 26% lower respectively than those caused by ascorbic acid. In contrast, sodium metabisulphite, failed to significantly enhance I-CYP binding above that level achieved in the presence of propranolol (10 μM). Thus, reducing activity is not the only property required of inducer agents. Binding may have involved the reduction of disulphide bonds, since ascorbic acid, DTT and L-cysteine can induce this reaction [7]. However, this phenomenon was apparently not associated with lipid peroxidation, since this is only significantly induced by ascorbate at concentrations greater than 10 μM [8]. Furthermore, ferric and ferrous ions (10 μM) which induce lipid peroxidation and manganese and cobalt ions which inhibit this reaction [9], failed to significantly alter ascorbic acid-induced I-CYP binding ($P > 0.1$; Student's non-paired *t*-test).

In the absence of ascorbic acid, light microscopic autoradiography revealed high densities of specific (i.e. propranolol-sensitive) I-CYP binding to beta-adrenoceptors in the epithelium and smooth muscle layers of guinea-pig trachea (Fig. 2b). In the presence of propranolol (10 μM), ascorbic acid and DTT caused marked increases in the densities of autoradiographic grains derived from I-CYP binding to non-beta-adrenoceptor sites. This binding was predominantly localized to the epithelium-submucosa interface (Fig. 2d, e).

Inhibitors of binding. Isoprenaline has previously been shown to reduce ascorbic acid-induced I-CYP binding to background levels [6]. The inhibitory activity of drugs from several different pharmacological groups, was also determined. At a concentration of 10 μM, the $beta_2$-selective agonist fenoterol (Boehringer, Ingelheim, Australia), 5-hydroxytryptamine, the alpha and beta-adrenoceptor agonists adrenaline and noradrenaline (Sigma) and the alpha-adrenoceptor antagonist phentolamine (Ciba-Geigy) caused 100% inhibition of binding at this concentration, while dopamine reduced binding by 86%. Conversely, 3-O-methylisoprenaline (Victorian College of Pharmacy), histamine (Sigma), nicotine (BDH), atropine (DHA), cimetidine (SKF), mepyramine (M & B), haloperidol (Searle), atenolol, propranolol, ICI-118551 (ICI) and methysergide (Sandoz Ltd., Switzerland) were all virtually inactive. Thus, ascorbic acid-induced I-CYP binding did not involve a previously characterized drug receptor type. Most importantly, non-radiolabelled cyanopindolol (CYP; Sandoz) also failed to inhibit ascorbic acid-induced I-CYP binding, suggesting that perhaps the iodine moiety of I-CYP was critical to the binding phenomenon.

^{125}I ion exists as an impurity of <5% i.e. about 2.5 pM in 50 pM I-CYP (Amersham). It is possible that ^{125}I ion rather than I-CYP was bound in the presence of ascorbic acid, DTT and L-cysteine. Exposure of guinea-pig tracheal sections to 2.5 pM NaI^{125} for 2 hr in the presence of ascorbic acid, resulted in levels of binding that were only about 5% of those achieved with 50 pM I-CYP. In contrast, when the concentration of NaI^{125} was increased to 50 pM, binding increased to levels that were comparable to those achieved

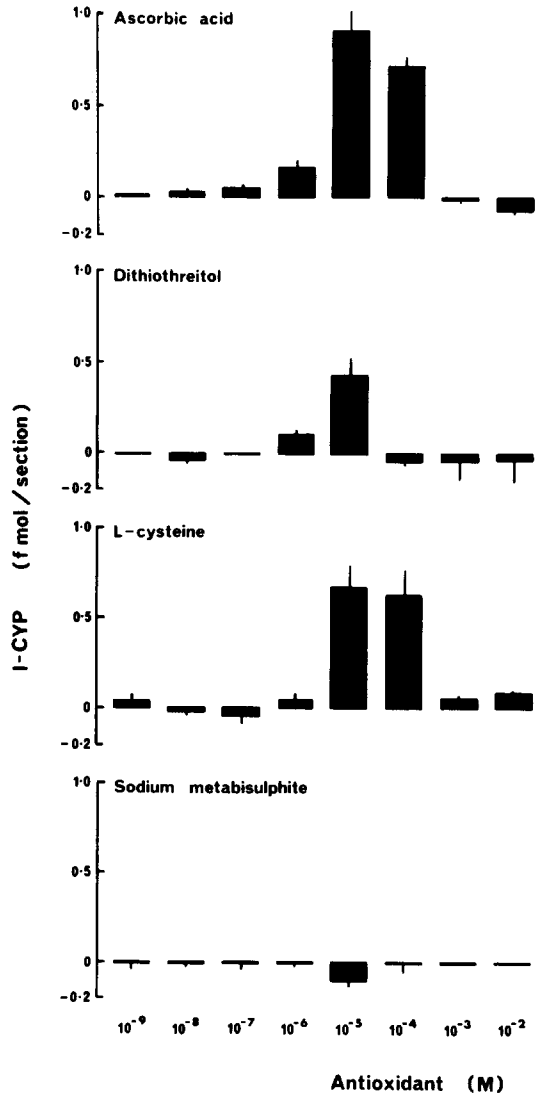

Fig. 1. Concentration-dependence of antioxidant-enhanced I-CYP (50 pM) binding to non-beta-adrenoceptor sites in guinea-pig trachea. Vertical lines represent standard errors of the means of at least 3 observations.

with 50 pM I-CYP. Ascorbic acid may significantly increase the concentration of free ^{125}I ion in solution by cleaving it from I-CYP. This possibility was tested by combining 50 pM I-CYP and 10 μM ascorbic acid in the presence and absence of slide-mounted tissue sections, as in binding studies and subjecting aliquots (100 μl) of these mixtures, as well as aliquots of I-CYP and of NaI^{125} to silica gel based ascending thin-layer chromatography. The solvent system used was ethyl acetate, isopropanol, ammonia and double distilled water (45:35:5:10). Chromatograms were air-dried and autoradiograms produced using sheets of X-ray film (Fuji RX-100). Radioactivity in developed regions was then quantified. No evidence of ascorbic acid-induced cleavage of ^{125}I from I-CYP was obtained. These data indicate that the iodine moiety of I-CYP was indeed critical for binding, but that both the free and the CYP-bound forms associated with non-beta-adrenoceptor sites.

This study has further characterized the anomolous binding of I-CYP to non-beta-adrenoceptor sites in guinea-pig trachea. These sites, which were predominantly localized

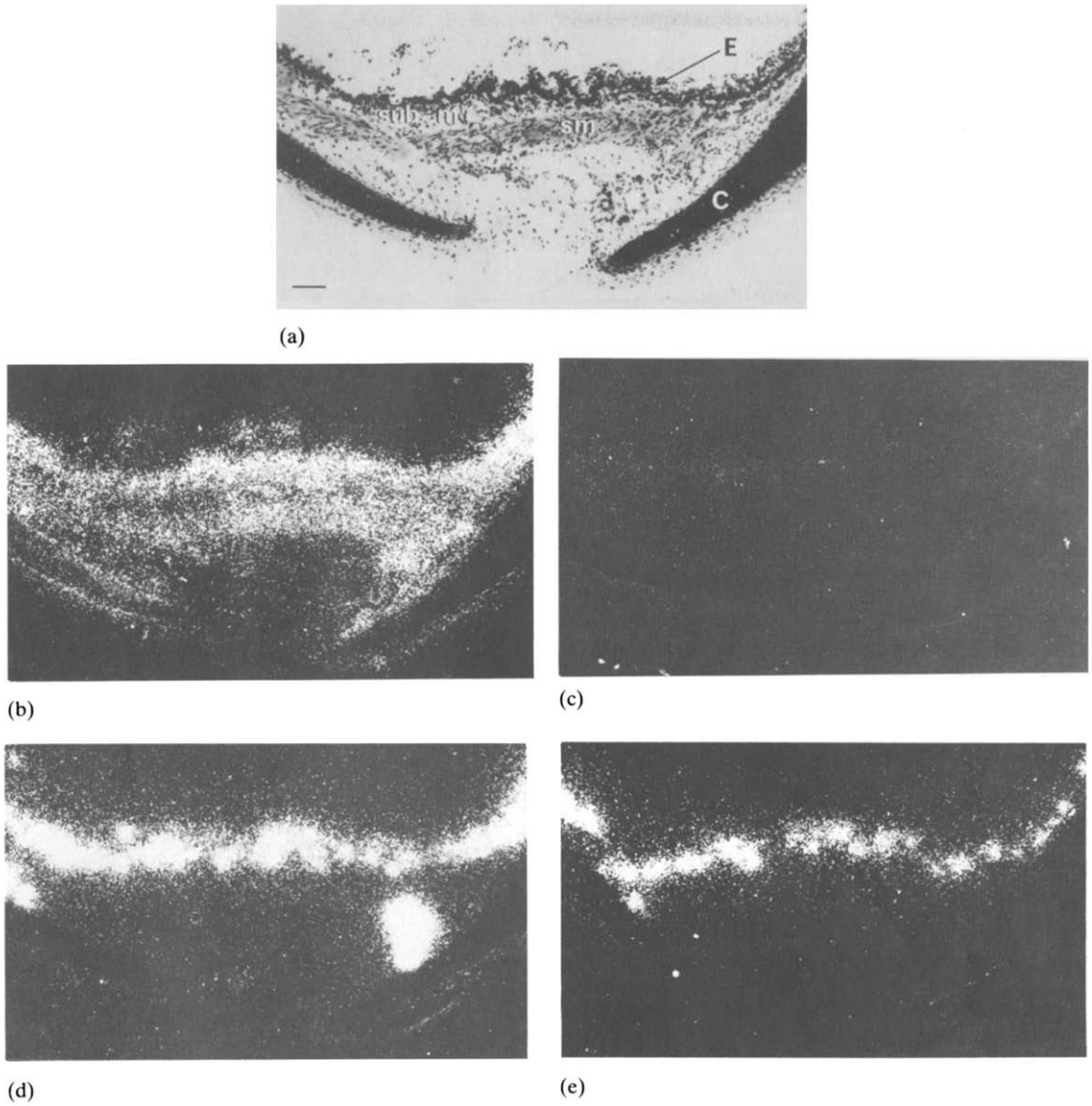

Fig. 2. (a) Light-field photomicrograph of a 10 μm frozen section of guinea-pig trachea, showing epithelium (E), airway smooth muscle (sm), sub-mucosa (sub-m) and cartilage (C). Dark-field photomicrograph of the above section showing the distribution of autoradiographic grains derived from I-CYP (50 pM) binding in the absence (b) and presence (c) of 10 μM propranolol. (d) in the presence of propranolol (10 μM) and ascorbic acid (10 μM) or (e) propranolol (10 μM) and dithiothreitol (10 μM).

at the interface of the epithelium and submucosa, were revealed in the presence of commonly used antioxidants including ascorbic acid, DTT and L-cysteine. The iodine moiety was apparently critical to the expression of this phenomenon. Clearly, investigations involving radioiodinated ligands must be conducted with caution, since levels of specific binding may be compromised by the effects of such antioxidants.

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