Inhibition by viozan of extravasation induced in rat trachea by capsaicin is mediated exclusively by β₂-adrenoceptors

Abstract The mechanism by which 2(3H)-benzothiazolone, 4-hydroxy-7-[2-[[2-[(2-phenylethoxy)propyl]sulphonyl]ethyl]amino]ethyl]-monohydrochloride (AR-C68397AA; viozan), a dual dopamine D₂/β₂-adrenoceptor agonist which has shown promise in the treatment of chronic obstructive pulmonary disease (COPD), inhibits the extravasation of plasma protein induced by capsaicin in the tracheas of Brown Norway rats has been re-evaluated.

Viozan (10–30 μg/kg given intratracheally; i.t.) inhibited dose-dependently the extravasation of plasma protein tagged with Evans Blue into rat trachea induced by capsaicin (10 μg/kg i.t.). Similar effects were seen with the selective β₂-adrenoceptor agonist, salbutamol (3–10 μg/kg i.t.), but the selective dopamine D₂ receptor agonist, quinagolide (10–30 μg/kg i.t.), was inactive. The effects of viozan and salbutamol were abolished by propranolol (3 mg/kg) given intraperitoneally (i.p.) but unaffected by sulphiride (3 mg/kg i.p.).

Thus, in contrast to claims in the literature, a functional response to dopamine D₂ receptor activation in a preclinical model of oedema arising from sensory nerve fibre activation in the rat lung could not be demonstrated. Moreover, no qualitative difference could be demonstrated between the response to a dual D₂/β₂-adrenoceptor agonist and a selective β₂-adrenoceptor agonist. The observations call into question whether a dual D₂/β₂-adrenoceptor agonist such as viozan would bring added benefit over established selective β₂-adrenoceptor agonists in the therapy of COPD.

Keywords Protein extravasation · Rat trachea · Capsaicin · Dopamine D₂ receptors · β₂-Adrenoceptors · Viozan · COPD

Introduction 2(3H)-Benzothiazolone, 4-hydroxy-7-[2-[[2-[(2-phenylethoxy)propyl]sulphonyl]ethyl]amino]ethyl]-monohydrochloride (AR-C68397AA; viozan) is a dual dopamine D₂/β₂-adrenoceptor agonist which in early clinical studies has shown promise in the treatment of chronic obstructive pulmonary disease (COPD; Newboll et al. 2001). It is reasoned that the addition of D₂ receptor agonism would bring advantages over selective β₂-adrenoceptor agonists by suppressing reflex activation of pulmonary sensory nerves leading to relief of dyspnoea and inhibition of mucous hypersecretion, cough and oedema arising from extravasation of plasma protein (Newboll et al. 2001). There is no evidence, however, that dopamine D₂ receptors are present on the sensory afferent fibres of human lung.

In contrast, dopamine D₂ receptors are present on sensory afferent fibres of the rat lung (Peiser and Fischer 2001) and evidence that viozan inhibits plasma protein extravasation induced by capsaicin in rat trachea by activation of D₂ receptors has appeared (Weyman-Jones et al. 1999). Moreover, two selective dopamine D₂ receptor agonists, a viozan analogue, AR-C65116AB, and ropinirole (Eden et al. 1991), have recently been shown to be effective in this model and inhibition was sensitive to blockade by the dopamine D₂ receptor antagonist, sulphiride (Trevisani et al. 2001).

We hereby confirm that viozan is indeed a potent suppressant of extravasation in rat trachea induced by capsaicin. However, contrary to the claims in the literature, the mechanism is entirely a consequence of β₂-adrenoceptor activation. Moreover, the potent and selective D₂ receptor agonist, quinagolide (Brownell 1996), was without effect in this model indicating that such sites are not functionally linked to the suppression of protein extravasation.
Materials and methods

Male Brown Norway rats weighing approximately 300 g were supplied by Biological Research Laboratories (Füllinsdorf, Switzerland). They were kept at an ambient temperature of 22±2°C under a 12-h normal phase light-dark cycle and fed on NAFAG pellets supplied by Nahr und Futtermittel (Gossau, Switzerland). Drinking water was freely available. All experiments were carried out with the approval of the Veterinary Authority of the City of Basel (Kantonales Veterinäramt, Basel-Stadt, Switzerland).

Protein extravasation into the trachea was measured by quantifying the amount of Evans Blue dye bound to plasma protein in the tissue. Rats were anaesthetised with thiopental sodium (70 mg/kg i.p.) and the left jugular vein cannulated. Evans Blue (30 mg/kg) was injected i.v. and 2 min later capsaicin (3–30 µg/kg) or an equivalent volume of saline (0.1 ml) was administered i.t. Ten minutes later, the tracheal wall was perfused in situ with phosphate-buffered saline (5 ml/min for 3 min) via a cannula inserted into the left ventricle with the descending aorta tied off. The trachea (from the larynx to the bifurcation) was removed, rinsed thoroughly in phosphate-buffered saline, blotted dry and weighed. Tissues were then incubated at 50°C in 2 ml formamide for 18–20 h and the Evans Blue concentration of the incubation fluid measured photometrically. The Evans Blue content of the trachea was expressed as ng/mg tissue wet weight.

Salbutamol, viozan, quinagolide or an equivalent volume of saline (0.1 ml) were administered i.t. 30 min before the capsaicin. Propranolol or sulpiride were administered i.p. 15 min prior to the agonists. Formamide and Evans Blue were obtained from Merck (Darmstadt, Germany). Drugs used were: thiopental sodium (Abbott, Baar, Switzerland); salbutamol, propranolol hydrochloride and S(-)-sulpiride (Sigma-Aldrich, Schnelldorf, Germany); 2(3H)-benzothiazolone-4-hydroxy-7-[2-[3-(2-phenylethoxy)propyl]sulphonyl]-ethyl]amino-ethyl]-monohydrochloride (AR-C68397AA; viozan) and quinagolide (synthesised in the laboratories of Novartis Pharma). Spectral characterisation of viozan was consistent with that reported by AstraZeneca (Eleye 2000). Final dilutions of all compounds were in normal saline and doses refer to the base forms.

Results

Capsaicin (3–30 µg/kg i.t.) induced dose-related increases in the Evans Blue content of the trachea (Fig. 1). A dose of 10 µg/kg, which produced a just submaximal response, was chosen for the agonist studies. Viozan (10 µg/kg and 30 µg/kg i.t.) given 30 min prior to capsaicin induced dose-dependent inhibition of the extravasation response (Fig. 2). Salbutamol (3 µg/kg and 10 µg/kg) also inhibited the response to capsaicin dose-dependently but quinagolide (10 µg/kg and 30 µg/kg) was without significant effect (Fig. 2).

Neither propranolol (3 mg/kg i.p.) nor sulpiride (3 mg/kg i.p.) affected the response to capsaicin per se (data not illustrated). Pretreatment with propranolol fully inhibited the response to just maximal doses of both salbutamol (10 µg/kg) and viozan (30 µg/kg). In contrast, responses to salbutamol and viozan were completely unaffected by pretreatment with sulpiride (Fig. 2).

Discussion

Extravasation induced by capsaicin is a standard method of quantifying the consequences of sensory nerve activation in the trachea (Geronpré et al. 1995). The fact that viozan suppressed tracheal extravasation induced by capsaicin in rats pretreated with propranolol was used to support the case for the added benefit of a combined dopamine D2/D3-adenoreceptor agonist over a selective β2-adenoreceptor agonist in the treatment of COPD (Newbold et al. 2001). The present data confirm the potent inhibitory effects of viozan on tracheal extravasation induced by capsaicin. However, contrary to the literature findings, the effect is entirely a consequence of β2-adenoreceptor activation and agonist activity at dopamine D2 receptors plays no role in the response.

Thus, the effect of viozan was mimicked closely by low doses of the selective β3-adenoreceptor agonist, salbutamol. Second, responses to both salbutamol and viozan were fully blocked by propranolol. Third, neither agonist was blocked by sulpiride at a dose (3 mg/kg) supramaximal for blockade of peripheral dopamine D2 receptors in vivo (Mueller et al. 1979). Finally, the selective D2 receptor agonist, quinagolide, which is fivefold more potent than viozan at the presynaptic dopamine D2 receptors in mouse vas deferens (unpublished observations), had no effect on capsaicin-induced extravasation at a dose (30 µg/kg) at which viozan was maximally effective. The fact that β2-adenoreceptor activation results in suppression of capsaicin-induced plasma protein extravasation is unsurprising; there are several examples in the literature where such activity has been shown (Advenier et al. 1992; Bolton and McDonald 1997). The fact that the D2 receptor agonist property of viozan does not manifest as inhibition of the response to capsaicin in the present model was unexpected based on the published findings (Weyman-Jones et al. 1999).

We have no plausible explanation for why the present data differ so fundamentally from the literature findings.