The β -adrenoceptor antagonist carteolol and its metabolite 8-hydroxycarteolol have different intrinsic sympathomimetic activities

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We have tested the effects of carteolol and 8-hydroxycarteolol on cAMP generation in S49 lymphoma and BC₃H₁ smooth muscle-like cells. Carteolol was a high affinity β -adrenoceptor antagonist in both systems but did not stimulate cAMP accumulation. The metabolite 8-hydroxycarteolol also was a high affinity antagonist. In contrast to its parent compound, however, it possessed an agonistic component which was considerably stronger than that of other β -adrenoceptor blockers with intrinsic sympathomimetic activity, e.g. dichloroisoprenaline, pindolol, or celiprolol. These results suggest that metabolites can possess different pharmacodynamic properties in terms of β -adrenoceptor interaction relative to parent compounds.

Keywords carteolol intrinsic sympathomimetic activity β -adrenoceptor

Introduction

Some β -adrenoceptor antagonists not only mitigate the response to agonists but also themselves have weak agonistic effects. This property has been termed 'intrinsic sympathomimetic activity' (ISA). Whereas β -adrenoceptor agonists are generally assumed to act by stimulation of adenylyl cyclase, it has been difficult to detect any enhancement of cAMP generation by β -adrenoceptor antagonists with ISA (Golf & Hansson, 1986; Jasper *et al.*, 1988; Michel *et al.*, 1987; Reynolds & Molinoff, 1986). We have recently developed an *in vitro* biochemical technique for measuring cAMP accumulation in response to β -adrenoceptor blockers with ISA (Jasper *et al.*, 1988). This technique utilizes the diterpene forskolin to amplify adenylyl cyclase stimulation in cultured cells and thereby elicits weak stimulation of adenylyl cyclase activity by β -adrenoceptor blockers with ISA. Carteolol is a novel β -adrenoceptor antagonist which shows signs of ISA *in vivo*; *in vitro*, however, carteolol may not have ISA (Sander & Giles,

1986). Therefore, we studied the partial agonistic activity of carteolol and its major metabolite 8-hydroxycarteolol in cultured cells.

Methods

Wild-type (strain 24.3.2) S49 lymphoma cells were maintained in suspension culture as described (Darfler *et al.*, 1982). BC₃H₁ smooth muscle-like cells were grown as described (Hughes & Insel, 1986) and experiments were conducted after 4–6 days of confluence ($\approx 10^6$ cells/35 mm dish). S49 cells were incubated at 37° C for 5 min with either carteolol or 8-hydroxycarteolol in the presence or absence of 1 μ M forskolin, and intracellular cAMP accumulation was assayed as described previously (Jasper *et al.*, 1988). A slightly modified procedure was used with BC₃H₁ cells: prior to each experiment, the culture medium was aspirated from the plates, and the cells were incubated in Dul-

Figure 1 Cyclic AMP accumulation in S49 lymphoma cells. Shown are the concentration-response curves for carteolol in the presence of 1 μ M forskolin ($\blacklozenge$) and 8-hydroxycarteolol in the presence ($\blacksquare$) and absence of forskolin ($\square$); maximal stimulation by isoprenaline (1 μ M) is shown in the bar graph for comparison. Basal and forskolin-stimulated cAMP levels were subtracted, and data are displayed as the mean $\pm$ s.e. mean of 2–4 independent experiments.

becco's modified Eagle's medium (pH 7.4) supplemented with 20 mM HEPES and 1 mg ml⁻¹ bovine serum albumin at 37°C. After 30 min the media was aspirated and replaced by 1.8 ml fresh medium, and drugs were added. Stimulation of cAMP accumulation was terminated by aspiration of the medium and addition of 1 ml of ice-cold 'stop' buffer (5 mM potassium phosphate, 150 mM KCl, 0.5 mM isobutylmethylxanthine, and 2 mM EDTA, pH 6.8) to each plate as described by Meier *et al.* (1985). Plates were kept on ice and scraped with a plastic spatula. Cells were then transferred to microfuge tubes, and the microfuge tubes placed in boiling water for 5 min. The generated cAMP was measured as described (Jasper *et al.*, 1988).

Results

In S49 lymphoma cells, carteolol antagonized 0.1 μ M (–)isoprenaline-stimulated cAMP accumulation with a pK_i of 8.79 ± 0.38 ($n = 3$) but by itself did not elevate intracellular cAMP levels in concentrations up to 10⁻⁵ M, even in the presence of 1 μ M forskolin (Figure 1). In contrast, the metabolite 8-hydroxycarteolol stimulated cAMP accumulation in S49 cells in the presence (pEC₅₀ 8.00 ± 0.26 , $n = 3$) and even in the absence (pEC₅₀ 7.24 ± 0.13 , $n = 2$) of 1 μ M forskolin (Figure 1). Maximal stimulation by 8-hydroxycarteolol in the absence of forskolin was 15% of that produced by 1 μ M isoprenaline.

The pEC₅₀ values for 8-hydroxycarteolol-stimulated cAMP accumulation in the absence and presence of forskolin were similar to the pK_i value for inhibition of 0.1 μ M isoprenaline-stimulated cAMP accumulation (7.84 ± 0.37 , $n = 3$, determined in the absence of forskolin). The stimulation of cAMP generation by 8-hydroxycarteolol in the presence of forskolin was completely abolished by 1 μ M (–)-propranolol (data not shown), thereby confirming that this effect was mediated by β -adrenoceptors.

In BC₃H₁ smooth muscle-like cells carteolol also blocked isoprenaline-stimulated cAMP accumulation but did not itself significantly increase intracellular cAMP in concentrations up to 10⁻⁵ M (data not shown). Similar to the results found in S49 cells, 8-hydroxycarteolol stimulated cAMP accumulation in the absence and presence of forskolin (data not shown).

Discussion

Our data show that carteolol is a potent β -adrenoceptor antagonist. However, carteolol does not stimulate cAMP accumulation in S49 and BC₃H₁ cells whether forskolin is present or not. As other drugs with ISA (e.g., pindolol, celiprolol, or dichloroisoprenaline) stimulate cAMP accumulation in S49 cells in the presence of forskolin (Jasper *et al.*, 1988), our results suggest that carteolol itself either lacks ISA or that if it possesses ISA, this activity is weaker than that of pindolol, celiprolol, and dichloroisoprenaline.

Because carteolol shows signs of ISA *in vivo*, we tested the carteolol metabolite 8-hydroxycarteolol which is known to have ISA properties (Sander & Giles, 1986). 8-hydroxycarteolol, like carteolol, was also a potent β -adrenoceptor antagonist. In contrast to its parent compound, however, 8-hydroxycarteolol stimulated cAMP generation. This effect could be demonstrated in either the absence or presence of forskolin. Because other β -adrenoceptor antagonists with ISA do not measurably induce cAMP generation in these cells in the absence of forskolin (Jasper *et al.*, 1988), we conclude that the agonistic activity of 8-hydroxycarteolol is considerably stronger than that of pindolol, celiprolol, or dichloroisoprenaline. Based on our data, 8-hydroxycarteolol should be classified as a partial agonist with an intrinsic efficacy of 15% compared with isoprenaline.

It should prove of interest to test other parent compounds and metabolites in our *in vitro* assay system to determine if the *in vivo* ISA of other

β -adrenoceptor blockers is also caused by their metabolites. From a clinical perspective the current findings raise the intriguing possibility that drugs or disease states which affect carteolol metabolism might alter not only the pharmacokinetic but also the pharmacodynamic profile of the drug in terms of β -adrenoceptor-mediated actions, such as effects on heart rate, lipid metabolism, or β -adrenoceptor regulation.

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