Characterization of CHO-K1 Cells Stably Expressing PDE-IV Enzymes

Whole-Cell cAMP Determinations vs Broken-Cell Enzymatic Assays

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ABSTRACT

A CHO-K1 cell line stably expressing a recombinant full-length human PDE-IVa (HSPDE4A4B) enzyme was established under hygromycin B selection. Full-length expression of the protein was determined by Western blot analysis, which revealed the presence of a 125-kDa immunoreactive band using rabbit anti-PDE-IVa antibodies. The potency of inhibitor compounds was examined by their ability to increase cAMP in the whole-cell, and by their ability to inhibit cAMP hydrolysis in a 100,000g supernatant (soluble enzyme preparation) obtained from the same cell line. Inhibition of the expressed PDE-IVa activity by selective PDE-IV inhibitors—(R) and (S)-rolipram, RS 14203, and CDP 840—at 100 nM substrate demonstrated that RS 14203 and CDP 840 were the most potent with IC50 = 9 nM, followed by (R)-rolipram (IC50 = 110 nM) and (S)-rolipram (IC50 = 420 nM). The rank order of potencies of the inhibitors in elevating cAMP

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Cell Biochemistry and Biophysics 159 Volume 29, 1998
in the whole-cell assay was quite different from that on the soluble enzyme. RS 14203 was still the most potent compound in elevating cAMP. Moreover, the relative rank order of potencies between CDP 840 and (R)-rolipram changed dramatically, such that (R)-rolipram was more potent than CDP 840 = (S)-rolipram. An apparent 30-fold stereoselectivity between (R)- and (S)-rolipram was also noted. The whole-cell rank order of potencies was also maintained when PKA activity ratios were measured in place of cAMP levels. The ability of the compounds to elevate cAMP in the stable CHO-K1 cells appeared to track better with the potency of the compounds against the high-affinity (Sr) conformer of the enzyme rather than the low-affinity catalytic state.

INTRODUCTION

The low-$K_m$ cAMP dependent Type IV phosphodiesterase (PDE-IV) family of enzymes has recently generated much interest as potential biochemical targets for the treatment of bronchial asthma and inflammatory disease in humans. This new-found enthusiasm arises from in vitro data demonstrating that potent and selective inhibitors of the PDE-IV enzymes will inhibit the activation of proinflammatory cells (1). Additionally, data obtained from in vivo experiments show that allergen-induced bronchospasm and airways inflammation are attenuated in allergic animals pretreated with potent bioavailable PDE-IV inhibitors (2,3). In the process of establishing both recombinant PDE-IV-based enzymatic assays and whole-cell assays utilizing guinea pig eosinophils, we have observed that the rank order of potencies for a series of structurally diverse compounds did not correlate between their ability to elevate cAMP in the cell-based assay and their ability to inhibit the recombinant enzyme. In order to develop more potent and selective inhibitors of the PDE-IV enzyme, an understanding of the differences between the two assays was required. Since the observed differences between the two assays may, in part, be owing to differences in the recombinant PDE-IVa enzyme compared to that expressed by the guinea pig eosinophil (primary sequence, posttranslational modification) or that the guinea pig eosinophil harbors more than one of the four known PDE-IV isoforms, the objective of this study was to establish a mammalian cell line stably expressing the PDE-IVa enzyme. This stable cell line enables us to characterize the expressed PDE-IVa enzyme activity and to compare the ability of compounds to elevate...