Characterization of Bradykinin Receptors in Canine Cultured Corneal Epithelial Cells: Pharmacological and Functional Studies

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摘要

Abstract

The pharmacological properties of bradykinin (BK) receptors were characterized in canine cultured corneal epithelial cells (CECs) using [(3)H]-BK as a radioligand. Analysis of binding isotherms gave an apparent equilibrium dissociation constant of 0.34 +/- 0.07 nM and a maximum receptor density of 179 +/- 23 fmol/mg protein. Neither a B(1) receptor-selective agonist (des-Arg(9)-BK) nor antagonist ([Leu(8), des-Arg(9)]-BK) significantly inhibited [(3)H]-BK binding to CECs, thus excluding the presence of B(1) receptors in canine CECs. The specific binding of [(3)H]-BK to CECs was inhibited by B(2) receptor-selective agonists (BK and kallidin) and antagonists (Hoe 140 and [D-Arg(0), Hyp(3), Thi(5,8), D-Phe(7)]-BK), with a best fit using a one-binding-site model. The order of potency for the inhibition of [(3)H]-BK binding was BK = Hoe 140 > kallidin > [D-Arg(0), Hyp(3), Thi(5,8), D-Phe(7)]-BK. Stimulation of CECs by BK produced a concentration-dependent accumulation of inositol phosphates (IP) and an initial transient peak of intracellular Ca(2+). B(2) receptor-selective antagonist ([D-Arg(0), Hyp(3), Thi(5,8), D-Phe(7)]-BK) significantly antagonized the BK-induced responses with dissociation constants of 6.0-6.1. Pretreatment of CECs with pertussis toxin (PTX) or cholera toxin did not alter the BK-induced IP accumulation. Incubation of CECs in the absence of external Ca(2+) led to a significant attenuation of the IP accumulation induced by BK. These results demonstrate that BK directly stimulates phospholipase C-mediated signal

transduction through BK B(2) receptors via a PTX-insensitive G protein in canine CECs. This effect may function as the transducing mechanism for BK-mediated cellular responses.