Identification of a B2-bradykinin receptor linked to phospholipase C and inhibition of dopamine stimulated cyclic AMP accumulation in the human astrocytoma cell line D384

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Summary. We have examined the activation of a phospholipase C signal transduction pathway by a B2-bradykinin receptor in the human astrocytoma cell line D384 and how this influences D1-dopamine receptor stimulated cyclic AMP accumulation. Addition of bradykinin to D384 cells resulted in a concentration-dependent (10⁻¹⁻¹⁻¹⁰⁻⁶ M) increase in the accumulation of [³H]inositol phosphates and a similar concentration-dependent transient increase in specific [³H]β-phorbol-12,13-dibutyrate binding which is indicative of translocation of protein kinase C from the cytosol to the membrane. Changes in intracellular Ca²⁺ of single cells, measured using the fluorescent indicator dye fura-2, indicated that bradykinin produced a rapid, but transient, increase in intracellular calcium. The Ca²⁺ response was largely independent of extracellular Ca²⁺ supporting the idea that receptor activation leads to mobilization of Ca²⁺ from intracellular stores. However, extracellular Ca²⁺ was required for a response to a rechallenge with bradykinin. The bradykinin B₂-receptor agonist kallidin increased cytosolic Ca²⁺ in a similar manner to bradykinin. The Ca²⁺ response to bradykinin could be partially reduced in the presence of the B₂-receptor antagonist [{D-Arg°-Hyp, D-Phe⁷, β-(2-Thienyl)-Ala⁵,⁸}]-bradykinin, whereas the B₁-receptor agonists [Des-Arg⁹]-bradykinin and [Des-Arg¹⁰]-kallidin were ineffective. Bradykinin was also found to attenuate dopamine stimulated cyclic AMP accumulation in D384 cells, at similar concentrations previously observed to stimulate the phospholipase C signal transduction pathway, in the presence of the phosphodiesterase inhibitor, rolipram. In contrast, no attenuation was observed in the presence of the phosphodiesterase inhibitor 1-isobutyl 3-methylxanthine, although the level of dopamine stimulated cyclic AMP observed was lower than in the presence of rolipram. Furthermore, the effect of bradykinin could be mimicked by a calcium ionophore, but not a phorbol ester. These data suggest that D384 cells express a B₂-bradykinin receptor coupled to polyphosphoinositide-specific phospholipase C. Activation of this receptor results in elevated levels of cytosolic Ca²⁺ and to a reduction of D₁-dopamine receptor stimulated cyclic AMP accumulation.

Key words: B₂-Bradykinin receptor — Intracellular calcium — Phorbol ester binding — Cyclic AMP — D₁-dopamine receptor — Phosphodiesterase

Introduction

Bradykinin is involved in a variety of biological processes including the regulation of blood pressure, pain, neurotransmission, inflammation and cell proliferation. These effects are mediated through at least two different receptors, termed bradykinin B₁ and B₂ receptors, which have been pharmacologically characterized using different kinin analogues with agonist and antagonist properties (Regoli et al. 1990). In many cell types, including the neuroblastoma-glioma hybrid NG108-15 (Ozaki et al. 1986), glioma C6-4-2 (Reiser et al. 1990) and astrocytoma 1321N1 cells (Hepler et al. 1987), bradykinin receptors activate phospholipase C mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate in the plasma membrane. The resultant increase in diacylglycerol and inositol 1,4,5-trisphosphate activate protein kinase C and release Ca²⁺ from intracellular stores (Nishizuka 1984; Berridge and Irvine 1989).

Previously we reported the isolation of a clone D384 from the human astrocytoma cell line G-CCM which expresses a dopamine receptor with a pharmacological profile typical of a mammalian striatal D₁-dopamine receptor (Balmforth et al. 1988). The human astrocytoma cell line D384 is a homogeneous cell population, expressing D₁-dopamine receptors, which can be grown in large numbers. In this cell line dopamine induced stimulation of cyclic AMP accumulation in intact D384 cells is up to
50 fold above basal compared to only two to three fold increases observed in striatal homogenates.

The aim of the present study was to identify a bradykinin receptor expressed by D384 cells coupled to the phospholipase C signal transduction pathway and to examine whether activation of this pathway could modulate D1-dopamine receptor stimulated cyclic AMP accumulation.

Materials and methods

Materials. Dopamine, propranolol, fura-2AM, bradykinin, 1-isobutyl-3-methylxantnine (IBMX), β-phorbol-12,13-dibutyrate (PD Bu), β-phorbol 12-myristate,13-acetate (TPA) and ionomycin were purchased from Sigma Chemicals (Poole, Dorset, England); [3H]Hadenine, adenosine [14C]cyclic AMP, [3H]PD Bu, and [3H]myo-inositol from New England Nuclear; 4-(3-cyclopentyloxybenzoyl)-1-piperazineethanesulphonic acid. Cells were then exposed to [3H]PD Bu (20 nM) for 5 min followed by bradykinin at the times and concentrations indicated in the figure legends. All assays were terminated by aspiration of the binding mixture, three rapid washes with serum free medium containing 20 mM Hepes (2 ml), and solubilising the cells with 1 M NaOH (1 ml). The samples were neutralized with 1 M HCl (1 ml) and assessed for tritium content using a LKB Rackbeta scintillation counter. Specific binding represents the difference between total binding and that measured in the presence of 10 μM unlabelled PD Bu.

Measurement of intracellular calcium. Cells subcultured on glass coverslips were incubated with the cell permeant fluorescent probe [2-AM (5 μM) in a Krebs-Ringer buffer (pH 7.4) consisting of 145 mM NaCl, 5 mM KCl, 1.3 mM MgCl2, 1.2 mM Na2HPO4, 1.3 mM CaCl2, 10 mM glucose, 20 mM Hepes and 0.1% bovine serum albumin, at 37°C for 20 min. Loaded cells were then washed free of extracellular dye and the coverslip mounted in an aluminium open culture chamber which was then placed on the stage of an inverted microscope (Zeiss Axiovert 35) and maintained at 37°C in Krebs-Ringer buffer (1.5 ml). The microscope was equipped for dual wavelength epifluorescence microfluorometry. A 40X Zeiss neofluor objective was used for all experiments. An adjustable external diaphragm was used to further limit the measuring field to 2—4 cells. The fluorescence of the measurement field alternatively excited at 340 and 380 nm, was measured at 510 nm with a photon counting photomultiplier tube; a Zeiss MSP 20 microscope system processor was used to control the process. The ratio of fluorescence emission intensity at 340 nm to that at 380 nm was recorded at 2 s intervals and converted to cytosolic Ca2+ concentrations on a point to point basis using the equation described by Grynkiewicz et al. (1985) and an apparent calcium dissociation constant value for fura-2 of 224 nM at 37°C (Grynkiewicz et al. 1985). Calibration of the system was performed according to Cohen et al. (1987).

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Materials and methods

Measurement of total inositol phosphates. The measurement of total inositol phosphates in D384 cells was based on a modification of the technique described previously by Dubyak et al. (1988). D384 cells were incubated in inositol free culture medium (2 ml) containing 0.5 mM EDTA (3.7 ml) and applied to an anion exchange column [2 ml of a 1:1 (vol/wt) mixture of water and Bio-Rad AG1-X8 resin (mesh, 100-200 formate form)]. [3H]myo-inositol and [3H]glycerophosphoinositol were removed from the column by washing three times with serum free medium containing 10 mM LiCl (2 ml). Cells were stimulated by adding 1 ml of bradykinin in the above medium for the times and in the concentrations indicated in the figure legends. Incubations were terminated by the addition of 1.5 M perchloric acid (0.5 ml). Samples (1.4 ml) were placed on ice and neutralised with 1 M HC1 (1 ml) and assessed for tritium content using a LKB Rackbeta scintillation counter.

Measurement of phorbol ester binding to intact cells. The measurement of phorbol ester binding to intact cells was based on a modification of the technique described previously by Trilivas and Brown (1989). Binding assays were conducted at 37°C in a total volume of 1.5 ml and were begun by replacing the medium with fresh serum free medium containing 20 mM Hepes [4-(2-hydroxyethyl)-1-