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Bradykinin inhibits potassium (M) currents in N1E-115 neuroblastoma cells

Responses resemble those in NG108-15 neuroblastoma × glioma hybrid cells

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Application of bradykinin to voltage-clamped N1E-115 mouse neuroblastoma cells evoked sequential outward and inward membrane currents, accompanied by an increase and decrease of membrane conductance, respectively. Methacholine produced an inward current with a decreased conductance. The outward current response to bradykinin was imitated by intracellular inositol 1,4,5-trisphosphate (IP_3). Bath application of phorbol dibutyrate induced an inward current and potentiated the response to IP_3. We conclude that the response of these cells to bradykinin is identical to that of NG108-15 hybrid cells, and therefore may be attributed to the dual effects of inositol trisphosphate and diacylglycerol formed by hydrolysis of phosphatidylinositide.

Bradykinin; Methacholine; Inositol trisphosphate; Membrane current; M-current; Neuroblastoma; (Mouse)

1. INTRODUCTION

The nonapeptide bradykinin (BK) produces a sequential hyperpolarization and depolarization in NG108-15 neuroblastoma × glioma hybrid cells [1,2]. The hyperpolarization is due to the activation of a Ca^{2+}-dependent K^+ current, probably resulting from the formation of inositol 1,4,5-trisphosphate (IP_3) and the subsequent release of intracellular Ca^{2+}, since it can be replicated by injections of Ca^{2+} and of IP_3 [3,4]. The subsequent depolarization is associated with a fall in membrane conductance, primarily due to the inhibition of a voltage-dependent K^+ current, I_M [4]. This latter change may be mediated by the coincidental formation of diacylglycerol, with consequent activation of protein kinase C [5], since this effect can be replicated and occluded by phorbol dibutyrate [4].

Recently, Tertoolen et al. [6] have reported that BK application to N1E-115 neuroblastoma cells produces a similar biphasic membrane potential change, but with the difference that the secondary depolarization appears to be accompanied by an increased membrane conductance instead of a decrease. Further, since injections of inositol 1,3,4-trisphosphate (IP_3-1,3,4) produced a small but apparently similar depolarization with associated increase in input conductance (in agreement with our previous observations on NG108-15 cells [7]), Tertoolen et al. [6] suggest that the secondary depolarization produced by BK in these

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cells is mediated by this inositol phosphate isomer rather than by diacylglycerol-induced C-kinase activation.

We now report some experiments where we have examined the action of BK on N1E-115 cells under voltage clamp. We show that these cells respond in an identical manner to NG108-15 cells, and therefore conclude that isomers of inositol trisphos-

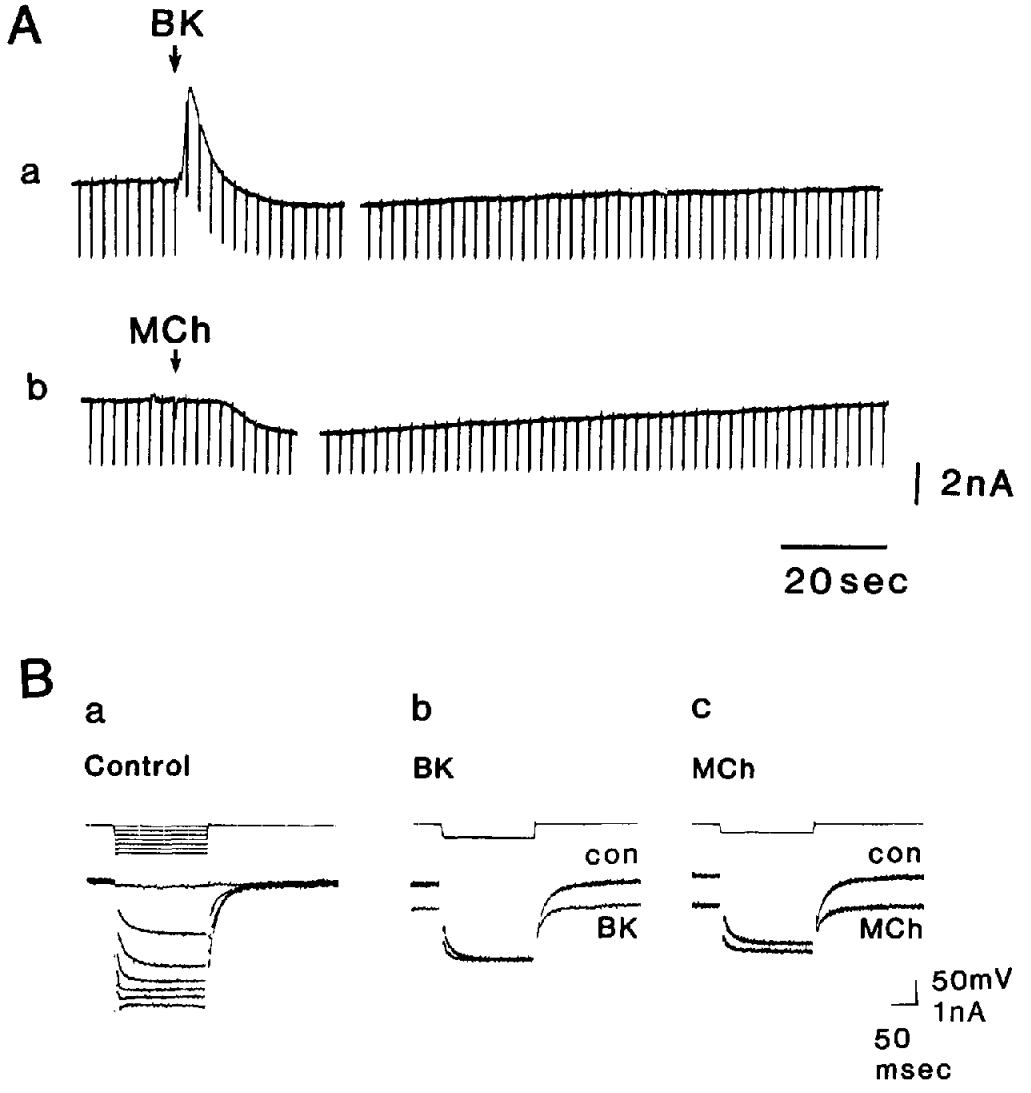

Fig. 1. Effects of bradykinin (BK; 2 μl, 10 μM pipette concentration) and methacholine (MCh; 2 μl, 200 mM pipette concentration) on a voltage-clamped N1E-115 neuroblastoma cell recorded at 35°C. (A) Membrane current changes recorded at the clamp holding potential of −30 mV (inward current downwards); downward deflections are inward current transients evoked by constant −30 mV (a) or −20 mV (b) 200 ms hyperpolarizing steps, to measure input conductance. (B) Oscilloscope records of inward current transients recorded in A. Upper trace, recorded voltage; lower traces, current. (a) Family of superimposed currents evoked by hyperpolarizing steps from −10 to −70 amplitude. (b) Superimposed records of currents produced by −30 mV steps before (con) and during (BK) the inward current produced by bradykinin in panel A,a. (c) Superimposed records of currents recorded before (con) and during (MCh) the inward current produced by methacholine in panel A,b. Note that the inward currents produced by both bradykinin and methacholine are accompanied by decreased evoked current amplitudes and a diminution in the amplitude of the time-dependent components of the current transients.

phate are unlikely candidates as mediators of the secondary depolarization.

2. MATERIALS AND METHODS

N1E-115 cells were grown in 35-mm culture dishes and differentiated with 2% DMSO as described [2]. Membrane current was measured by the single-electrode voltage-clamp method as in [4]. BK (2 μl, 1–100 μM in 150 mM NaCl) was applied to the cell surface through ejection pipettes. Cells were constantly perfused with Hepes-buffered (10 mM, pH 7.4) DMEM solution.

3. RESULTS

3.1. *Action of bradykinin*

Addition of BK to N1E-115 cells produced an initial (transient) outward current as shown in fig.1A. This is the voltage-clamp current equivalent to the hyperpolarization observed by Tertoolen et al. [6] in the same cell line. The outward current was associated with an increased conductance (2–5-fold), as measured by the current responses to transient voltage jumps.

Following the outward current, BK application evoked a secondary inward current, which was accompanied by a decrease in input membrane conductance (fig.1A). Inspection of the current transient produced by the voltage commands shows that this decrease in conductance was due primarily to inhibition of the slow current relaxations accompanying voltage jumps (fig.1B,b). As pointed out previously [7], these current relaxations are due to voltage-induced deactivation and reactivation of the voltage-dependent K^+ current,

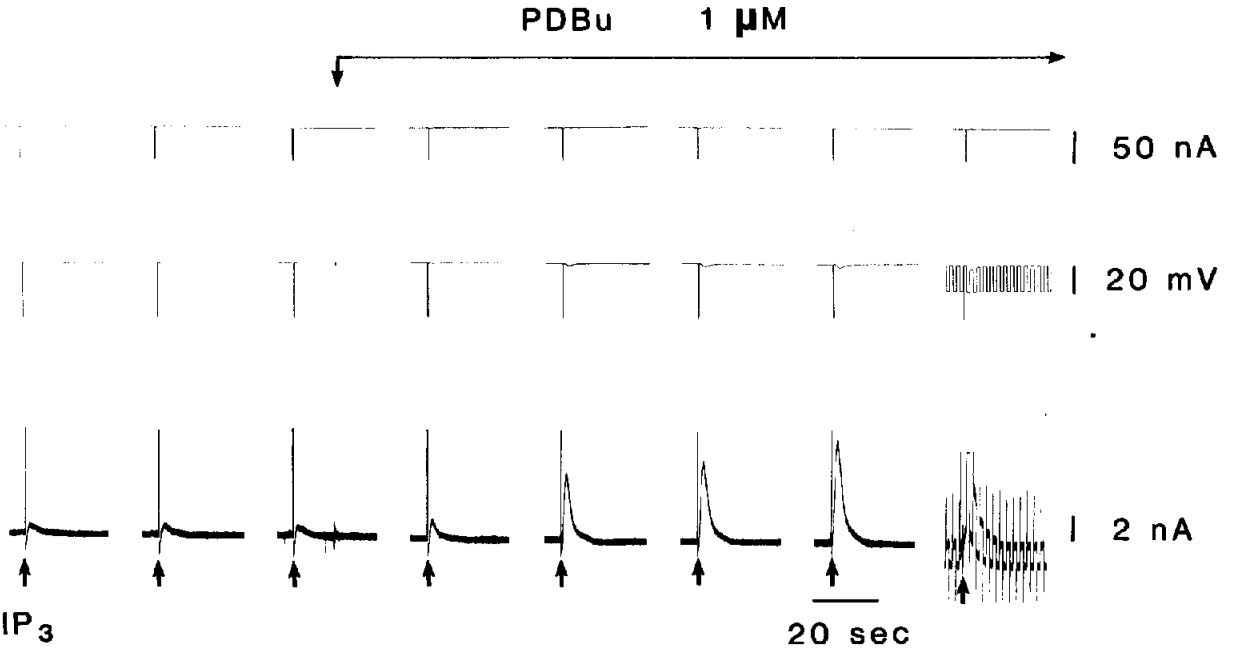

Fig.2. Outward current responses to intracellular iontophoretic injection of IP_3 (200 ms, −50 nA) at 2 min intervals into an N1E-115 cell voltage-clamped at −45 mV. Upper trace, iontophoretic current; middle trace, membrane voltage; lower trace, membrane current. (The iontophoretic current was not completely clamped, so that the injection led to a transient hyperpolarization; the subsequent outward current was not due to this hyperpolarization, however, because, in control experiments, no such outward current followed an equivalent injection of Cl^-.) During the last of the responses illustrated, 1 s, −20 mV voltage steps were superimposed to monitor changes in input conductance; the resultant current excursions were increased during the IP_3-induced outward current, indicating an increased conductance. Phorbol dibutyrate (PDBu, 1 μM) was added to the perfusion fluid at the time indicated. Note that PDBu strongly enhanced the response to IP_3 and produced a small net inward current. (The inward current is smaller than that produced by bradykinin or methacholine in fig.1 because the cell was clamped at a more negative holding potential, at which the M-current is less strongly activated, in order to amplify the outward current response to IP_3.)

I_M. In accordance with this, the relaxations shown in fig.1B (a) accelerated with increasing hyperpolarization and reversed between −75 and −85 mV, as expected for I_M deactivation [8]. Further, application of the cholinergic agonist, methacholine (which characteristically inhibits I_M in sympathetic neurons [8]), also inhibited these relaxations (fig.1B,c) and produced an associated inward current with a fall of conductance, precisely imitating the BK-induced secondary inward current.

Comparable effects of BK were recorded in representative cells in 6 cultures. One cell showed no clear conductance change during the inward current, but no cell showed an increased conductance.

3.2. *Effects of IP_3 and phorbol dibutyrate*

As in NG108-15 cells [4,7], and in agreement with Tertoolen et al. [6], intracellular iontophoresis of IP_3 induced an outward current resembling the initial responses to BK (fig.2). The iontophoretic currents required for these responses were comparable to those needed to evoke equivalent responses in NG108-15 cells [4]. Responses were well-sustained with injection intervals of 2 min.

Addition of phorbol dibutyrate (PDBu, 1 μM) to the perfusion fluid induced a small inward current, accompanied (where tested) with a fall in input conductance, and dramatically increased the amplitude of the outward currents produced by IP_3 injections (fig.2), in agreement with observations on NG108-15 cells [9].

4. DISCUSSION

The principal conclusion from these experiments is that the response of this sample of N1E-115 cells to BK was essentially identical to that we have previously noted in NG108-15 cells: namely, that there is a biphasic response consisting of an initial outward current with an increased conductance, followed by an inward current with a clear decrease in conductance, the latter primarily affecting the voltage-dependent M-conductance. We note particularly that these cells had well-developed M-currents and that these were also inhibited by methacholine, which thereby induced an inward current identical with the second phase of current induced by BK.

The initial outward current agrees with the results of Tertoolen et al. [6]. However, the secondary inward current clearly differs from that described by Tertoolen et al. [6], which was accompanied by an apparent increase in conductance: we have been quite unable to detect such an increased conductance in any of our cell samples showing a well-developed inward current. Since Tertoolen et al. [6] did not describe the ionic basis for this current, we are not able to explain the discrepancy. We note, however, that their cells were not voltage-clamped, so the natural rectification of the cell membrane during the depolarization might go some way toward the apparent increase in conductance. Experimentally, the principal difference between the two sets of experiments is that our cells were impaled with K^+ citrate-filled microelectrodes whereas Tertoolen et al. [6] used KCl-filled electrodes. The latter tend to 'Cl^- load' cells, so one possibility is that the release of Ca^{2+} might also activate a Cl^- current like that described in glandular cells [10], which could lead to an inward current (Cl^- efflux) in such cells, masking any effect of I_M inhibition. In citrate-impaled cells, with lower internal Cl^- concentrations, such a current would be smaller and outward and hence add to the outward K^+ current. Effects of I_M inhibition in ganglion cells can also be complicated by changes in Cl^- current when KCl-filled electrodes are used [11].

In agreement with Tertoolen et al. [6], we find that intracellular injection of IP_3 can replicate the initial outward current produced by BK: this is therefore compatible with the view that, as in NG108-15 cells [4], the initial outward current might well be mediated by this product of PIP_2 hydrolysis. We have not tested other inositol trisphosphates in N1E-115 cells but have no reason to suppose that their effects differ from those previously seen in NG108-15 cells [9] or in N1E-115 cells [6] since both reports concur in describing an inward current or depolarization accompanied by an increased conductance. However, since – in our experiments – this clearly differs in nature from the inward current produced by BK, we cannot agree with the suggestion [6] that IP_3-1,3,4 might mediate the inward current component of BK action. Instead, our tests with N1E-115 cells, while rather limited, suggest that

these cells respond in a similar way to NG108-15 cells to phorbol dibutyrate in terms of inward current generation and enhancement of the response to IP_3, and hence that a more likely pathway for generating the inward current is the adjuvent production of diacylglycerol from PIP_2 and consequent activation of protein kinase C, as previously proposed [4] for the response of NG108-15 cells.

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