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Electrophysiological responses to bradykinin and microinjected inositol polyphosphates in neuroblastoma cells

Possible role of inositol 1,3,4-trisphosphate in altering membrane potential

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Addition of bradykinin to mouse N1E-115 neuroblastoma cells evokes a rapid but transient rise in cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$). The $[Ca^{2+}]_i$ rise is accompanied by a transient membrane hyperpolarization, due to a several-fold increase in K^+ conductance, followed by a prolonged depolarizing phase. Pretreatment of the cells with a Ca^{2+}-ionophore abolishes the hormone-induced hyperpolarization but leaves the depolarizing phase intact. The transient hyperpolarization can be mimicked by iontophoretic injection of $IP_3(1,4,5)$ or Ca^{2+}, but not by injection of $IP_3(1,3,4)$, $IP_4(1,3,4,5)$ or Mg^{2+} into the cells. Instead, $IP_3(1,3,4)$ evokes a small but significant membrane depolarization in about 50% of the cells tested. Microinjected $IP_4(1,3,4,5)$ has no detectable effect, nor has treatment of the cells with phorbol esters. These results suggest that, while $IP_3(1,4,5)$ triggers the release of stored Ca^{2+} to hyperpolarize the membrane, $IP_3(1,3,4)$ may initiate a membrane depolarization.

Inositol trisphosphate; Inositol tetrakisphosphate; Ca^{2+}; Membrane potential; Bradykinin

1. INTRODUCTION

The receptor-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) yields at least two second messengers in stimulated cells: diacylglycerol, an activator of protein kinase C [1], and inositol 1,4,5-trisphosphate, $IP_3(1,4,5)$, which triggers the release of Ca^{2+} from internal stores [2]. In addition to $IP_3(1,4,5)$, stimulated cells also produce $IP_3(1,3,4)$ and $IP_4(1,3,4,5)$ via a pathway that involves a specific $IP_3(1,4,5)$-3-kinase and a polyphosphate 5-phosphatase [3,4]. Recent evidence suggests that $IP_4(1,3,4,5)$ is likely to be a mediator of increased Ca^{2+} influx across the plasma membrane [5], while the physiological role, if any, of $IP_3(1,3,4)$ remains to be determined.

In the present study we have used mouse N1E-115 neuroblastoma cells which exhibit pronounced electrophysiological responses to various Ca^{2+}-mobilizing neurohormones. We show that bradykinin elicits a marked biphasic membrane potential change. The first phase, a transient hyperpolarization is due to IP_3-dependent release of stored Ca^{2+} into the cytoplasm, while the subsequent depolarizing phase can be mimicked, at least in part, by microinjection of $IP_3(1,3,4)$.

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2. MATERIALS AND METHODS

N1E-115 cells were grown on circular cover glasses and induced to differentiate into mature sympathetic neurones with 1.5% DMSO, as described [6,7]. Inositol polyphosphates were prepared and purified by HPLC as in Irvine et al. [8]. The inositol phosphates were dissolved in H_2O at final concentrations of 0.5–2 mM, and the solutions poured into fine-tipped micropipettes for iontophoretic injections. A second microelectrode (3 M KCl filled, 20–50 MΩ) was used to measure membrane potential and resistance using conventional electrophysiological techniques [7,9]. The temperature of the bathing solution was maintained at 33 ± 1°C by means of a Peltier element.

Measurements of $[Ca^{2+}]_i$ were carried out using the fluorescent Ca^{2+}-indicator indo-1 (Molecular Probes), according to published procedures [10,11].

3. RESULTS AND DISCUSSION

3.1. *Effects of bradykinin, IP_3, Ca^{2+} and ionomycin*

Addition of bradykinin (2 μM) to N1E-115 cells loaded with the fluorescent Ca^{2+}-indicator indo-1 results in an immediate transient rise in $[Ca^{2+}]_i$ (fig.1A) even in the presence of excess EGTA (not shown), indicating the release of Ca^{2+} from internal stores. Addition of A23187 or ionomycin (1 μM) to the cells evokes a $[Ca^{2+}]_i$ rise that persists for at least 10 min (fig.1A), as expected for ionophores that tend to equilibrate external and intracellular Ca^{2+} compartments.

The bradykinin-induced $[Ca^{2+}]_i$ rise is accompanied by an immediate hyperpolarization (lasting 10–15 s) followed by a depolarizing phase, 5–10 mV in amplitude, that usually lasts for a few minutes (fig.1B). Similar biphasic responses to bradykinin have been reported for rat glioma cells [12] and for neuroblastoma × glioma NG108-15 hybrid cells [13]. The initial hyperpolarization in response to bradykinin is accompanied by a several-fold decrease in membrane resistance (fig.1B), consistent with activation of $[Ca^{2+}]_i$-dependent K^+ channels [13,14]. During the depolarizing phase there is again a decrease in membrane resistance (fig.1B), suggesting the opening of a distinct set of ion channels. As the membrane potential again reaches its resting level, the membrane resistance increases to its original value. This biphasic pattern of membrane potential changes was observed in all of the 90 cells tested.

Direct evidence for the notion that the initial hyperpolarization is mediated by IP_3(1,4,5)-dependent release of intracellularly stored Ca^{2+}, comes from microinjection experiments (fig.2).

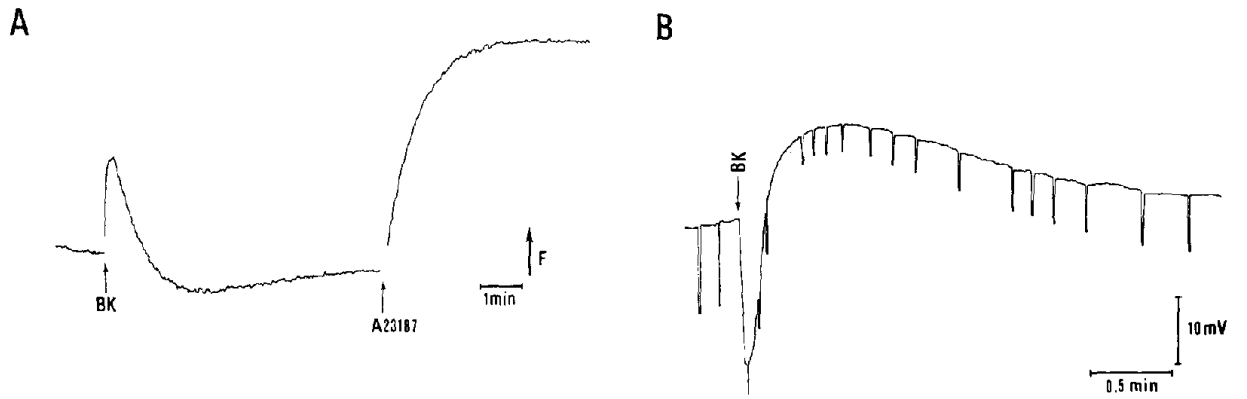

Fig.1. (A) Ca^{2+} sinals of indo-1 loaded N1E-115 cells in response to bradykinin (BK, 2 μM) and A23187 (1 μM). Cells were incubated with 2 μM indo-1 acetoxymethyl ester for 30 min at 37°C and $[Ca^{2+}]_i$-dependent fluorescence was monitored as described [11]. Resting $[Ca^{2+}]_i$ of N1E-115 cells is in the 150–200 nM range. Maximal indo-1 fluorescence corresponds to approx. 1–2 μM Ca^{2+}. (B) Typical electrophysiological recording of biphasic membrane potential change induced by bradykinin (2 μM). Membrane resistance was monitored by the voltage response to brief hyperpolarizing current injections (0.25 nA). Resting membrane potentials of N1E-115 cells are in the 35–45 mV range (interior negative).

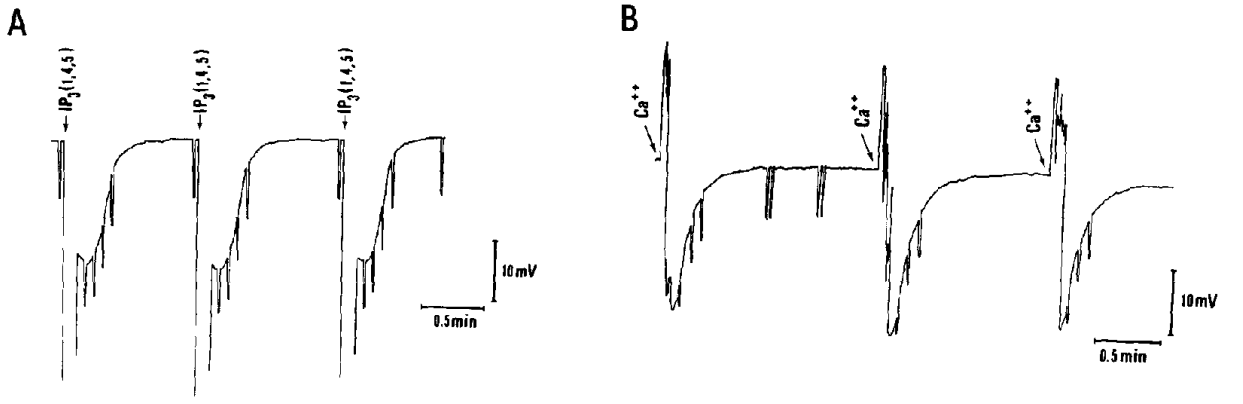

Fig.2. Membrane potential and resistance changes in single N1E-115 cells following microinjection of $IP_3(1,4,5)$ (A) and $CaCl_2$ (B). Injection current, 2.25 nA. Pipette concentrations: $IP_3(1,4,5)$, 2 mM; $CaCl_2$, 1 M. Other details as in fig.1B.

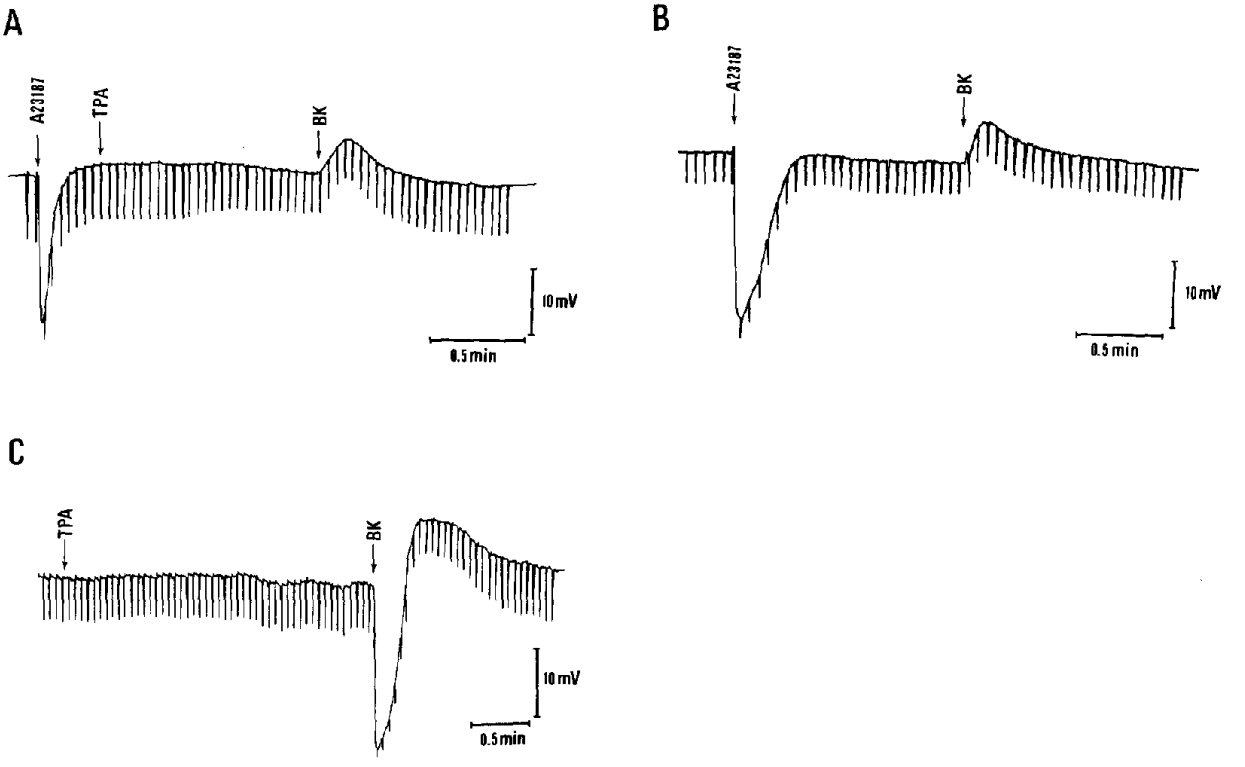

Fig.3. Membrane potential and resistance changes in N1E-115 cells following addition of the indicated stimuli. Final concentrations: A23187, 1 μM; TPA, 100 ng/ml; bradykinin, 2 μM. Other details as in fig.1B.

Iontophoretic injection of $IP_3(1,4,5)$ or Ca^{2+} into single cells evokes a marked transient hyperpolarization without any sign of a subsequent depolarizing phase (fig.2A,B). Microinjection of Mg^{2+} is without effect (not shown). Also, addition of A23187 or ionomycin close to an impaled cell evokes a transient hyperpolarization without a second depolarizing phase (fig.3A). When Ca^{2+} pools are equilibrated by pretreating the cells with ionophore for ~10–15 min the bradykinin-

induced hyperpolarization is completely blocked, but not the subsequent depolarization (fig.3A). From these results we conclude that, while the bradykinin-induced hyperpolarization is explained by $IP_3(1,4,5)$-triggered release of stored Ca^{2+}, the depolarizing phase is independent of $[Ca^{2+}]_i$ even when $[Ca^{2+}]_i$ is maintained at very high levels ($\sim 10\ \mu M$) using an ionophore.

Phorbol esters, such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and phorboldibutyrate (PdBu), which activate protein kinase C, produce a weak, depolarizing inward current in NG108-15 cells and thereafter inhibit the bradykinin-induced depolarization [15]. These findings led Higashida and Brown [15] to conclude that the depolarizing phase of the bradykinin response is attributable to kinase C closing specific K^+ channels. In N1E-115 cells, however, phorbol esters (TPA or PdBu, 100 ng/ml) fail completely to affect membrane potential and membrane conductance ([16] and results not shown); yet, these compounds do activate kinase C in N1E-115 quite readily, as judged from their effect on Na^+/H^+ exchange [16]. It is conceivable that kinase C might require a prior $[Ca^{2+}]_i$ rise to exert an effect on membrane potential and conductance. However, phorbol esters are still ineffective in cells in which $[Ca^{2+}]_i$ had already been raised by A23187 (fig.3B). Furthermore, under our experimental conditions the bradykinin-induced biphasic voltage response was not detectably affected by pretreating the N1E-115 cells with either TPA or PdBu (fig.3C). It therefore seems unlikely that the hormone-induced depolarizing phase in N1E-115 cells results from kinase C activation.

We also tested the possibility that cyclic nucleotides are involved in the initiation of the depolarizing phase. However, application of either 8-Br-cAMP or 8-Br-cGMP (1 mM) failed to elicit a detectable membrane potential change (not shown).

3.2. *Effect of $IP_3(1,3,4)$*

To determine whether inositol polyphosphates other than $IP_3(1,4,5)$ might contribute to the biphasic nature of the bradykinin response, we microinjected pure (prepared by HPLC) $IP_3(1,3,4)$ and $IP_4(1,3,4,5)$ at equivalent pipette concentrations. An intriguing effect is seen with $IP_3(1,3,4)$ (fig.4). After a small, initial hyperpolarization there is a distinct depolarizing phase, albeit less pronounced than usually seen with bradykinin. As with bradykinin, the depolarization is accompanied by a decreased membrane resistance. Such a response was observed in 55 of the 111 microinjection attempts. We presume that the $IP_3(1,3,4)$-induced hyperpolarization reflects the weak Ca^{2+}-mobilizing activity of this IP_3-isomer [8]. More importantly, we suggest that the subsequent depolarization is caused, either directly or indirectly, by the opening of an as yet unidentified ionic channel that may be responsible for the bradykinin-induced depolarizing phase.

3.3. *Effect of $IP_4(1,3,4,5)$*

Finally, we examined the possible effect of ion-

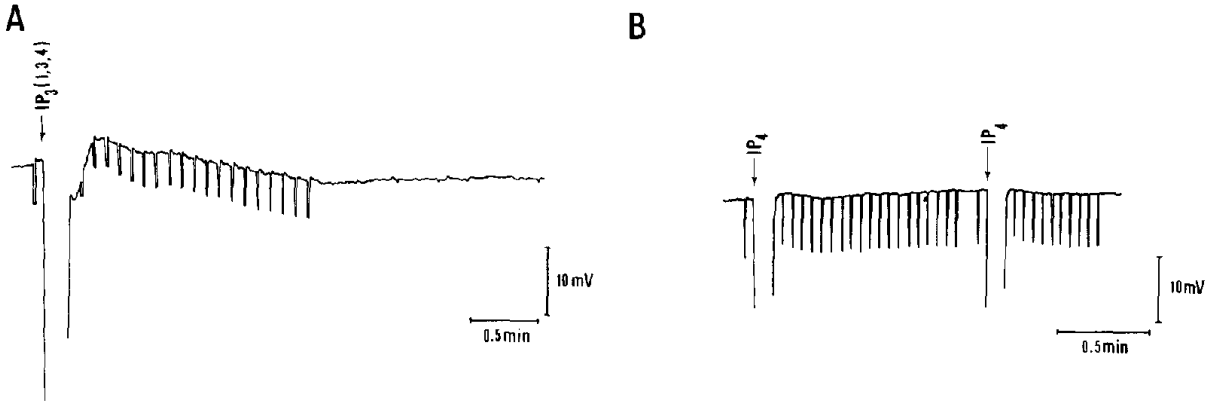

Fig.4. Typical electrophysiological responses to microinjected $IP_3(1,3,4)$ and $IP_4(1,3,4,5)$. Pipette concentrations of IP_3 and IP_4, 1–2 mM. Other details as in figs 1B and 2.

tophoretically injected $IP_4(1,3,4,5)$ in N1E-115 cells. As illustrated in fig.4B, the microinjection of $IP_4(1,3,4,5)$ (pipette concentration 0.5–2.0 mM) failed completely to evoke any detectable effect on membrane potential and resistance (29 microinjections in 10 different cells). This result agrees with the failure of $IP_4(1,3,4,5)$ to mobilize Ca^{2+} from internal stores [8], and it suggests that the local $IP_4(1,3,4,5)$ concentration is not high enough to yield sufficient $IP_3(1,3,4)$ (through dephosphorylation) to evoke the characteristic $IP_3(1,3,4)$ effect on membrane potential as in fig.4A. Presumably this observation also accounts for the failure of $InsP_3(1,4,5)$ to induce a depolarizing phase.

3.4. *Conclusions*

In conclusion, our proposed scheme for the action of bradykinin on N1E-115 cells differs from that suggested by Higashida and Brown [15] on NG108-15 cells, in that $IP_3(1,3,4)$ rather than kinase C is the primary candidate responsible for the depolarizing phase. Although the $IP_3(1,3,4)$-induced depolarization is smaller and less reliable than that evoked by bradykinin, this could simply be due to the localized accumulation of microinjected $IP_3(1,3,4)$ as opposed to an overall increase in $IP_3(1,3,4)$ levels just underneath the plasma membrane in hormonally stimulated cells.

When this manuscript was in preparation, Higashida and Brown [17] reported that microinjected $IP_3(1,3,4)$ and $IP_4(1,3,4,5)$ both evoke an inward (depolarizing) membrane current in NG108-15 cells, the nature of which remains to be identified. If this current is the same as that we observe here, then it seems likely that the effect of $IP_4(1,3,4,5)$ in NG108-15 cells [17] is due to its breakdown product $IP_3(1,3,4)$, because in our hands $IP_4(1,3,4,5)$, injected at the same concentration as $IP_3(1,3,4)$, had no effect on N1E-115 cells; a difference in IP_4 phosphatase levels between the two cell lines may be the explanation. However, according to Higashida and Brown, this $IP_3(1,3,4)$-induced current is not associated with the depolarizing phase of the bradykinin responses [15,17], which suggests more profound differences in physiology between the cell lines. Clearly, further study is required both to establish the suggested second messenger role of $IP_3(1,3,4)$ in hormone action, and to resolve the discrepancies in interpretation of the nature of the biphasic response to bradykinin.

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