The Monomeric G-Proteins Rac1 and/or Cdc42 Are Required for the Inhibition of Voltage-Dependent Calcium Current by Bradykinin

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Although regulation of voltage-dependent calcium current (\(I_{\text{Ca,V}}\)) by neurotransmitters is a ubiquitous mechanism among nerve cells, the signaling pathways involved are not well understood. We have determined previously that in a neuroblastoma-glioma hybrid cell line (NG108-15), the heterotrimeric G-protein G\(_{13}\) mediates the inhibition of \(I_{\text{Ca,V}}\) produced by bradykinin (BK) via an unknown mechanism. Various reports indicate that G\(_{13}\) can couple to RhoA, Rac1, and Cdc42, which are closely related members of the Rho family of monomeric G-proteins. We have investigated their role as signaling intermediates in the pathway used by BK to inhibit \(I_{\text{Ca,V}}\). Using immunoblot analysis and the PCR, we found evidence that RhoA, Rac1, and Cdc42 are expressed in NG108-15 cells. Intracellularely perfused recombinant Rho-GDI (an inhibitor of guanine nucleotide exchange specific for the Rho family) attenuated the inhibition of \(I_{\text{Ca,V}}\) by BK. These findings indicate that activation of RhoA, Rac1, or Cdc42 may be required for the response to BK. To determine whether any of these monomeric G-proteins mediate the response to BK, we have intracellularly applied blocking antibodies specific for each of the candidate proteins. Only the anti-Rac1 antibody blocked the response to BK. In parallel experiments, peptides corresponding to the C-terminal regions of Rac1 and Cdc42 blocked the same response. These data indicate a novel functional contribution of Rac1 and possibly also of Cdc42 to the inhibition of \(I_{\text{Ca,V}}\) by neurotransmitters.

Key words: G-proteins; calcium channels; neuropeptides; modulation; neuroblastoma-glioma; NG108-15; Rac1; Cdc42; G\(_{13}\)

The voltage-dependent calcium current (\(I_{\text{Ca,V}}\)) occupies a nodal position in neuronal communication, because it couples membrane depolarization to secretion of synaptic vesicles. The critical regulatory role of \(I_{\text{Ca,V}}\) is enhanced by the ubiquitous modulation of this current by a multitude of transmitter receptors (Tsien et al., 1991; Hille, 1992, 1994; Hescheler and Schultz, 1993; Hoffmann et al., 1994). Although in most cases, these transmitter actions are mediated via heterotrimeric G-proteins (Hepler and Gilman, 1992; Hamm and Gilchrist, 1996; Neer and Smith, 1996), the downstream signal transduction pathways leading to regulation of \(I_{\text{Ca,V}}\) often do not use the conventional second messengers (Hille, 1994). One signaling mechanism that might contribute to modulation of \(I_{\text{Ca,V}}\) by neurotransmitters involves sequential activation of heterotrimeric and monomeric G-proteins (Bourne et al., 1990, 1991). Activation of monomeric G-proteins would then provide the link for the recruitment of specific mitogen-activated protein kinase (MAPK) pathways (Hille, 1994; Cano and Mahadevan, 1995; Cobb and Goldsmith, 1995; Hunter, 1995; Bokoch, 1996; Kyriakis and Avruch, 1996).

Monomeric G-proteins coupled to MAPK pathways mediate many of the actions of growth factors in a variety of undifferentiated or transformed cells. Receptors for growth factors do not couple to heterotrimeric G-proteins for downstream signaling. Rather, they use tyrosine phosphorylation to initiate a chain of events leading to recruitment of monomeric G-proteins (Cobb and Goldsmith, 1995; Kyriakis and Avruch, 1996). The ensuing activation of MAPK pathways has a long-lasting impact on cellular proliferation and differentiation. Recent studies have enriched this scheme in various ways. For example, neurotransmitter receptors coupled to heterotrimeric G-proteins have been found to contribute to the activation of monomeric G-proteins (Cobb and Goldsmith, 1995; Bokoch, 1996; Kyriakis and Avruch, 1996), leading to downstream actions with speed comparable to that of conventional second messengers (Zhong, 1995; Hooley et al., 1996). In addition, monomeric G-proteins coupled to MAPK pathways have also been reported to play signaling roles in differentiated cells (Cobb and Goldsmith, 1995; Kyriakis and Avruch, 1996).

Using the NG108-15 neuronal cell line as a model system (Hamprecht et al., 1985), we have tested the hypothesis that monomeric G-proteins contribute to the regulation of \(I_{\text{Ca,V}}\) by neurotransmitters. In these cells, bradykinin (BK) and Leu-Enkephalin (Leu-Enk) inhibit the \(\omega\)-conotoxin-sensitive component of \(I_{\text{Ca,V}}\) via the heterotrimeric G-proteins G\(_{13}\) and G\(_{\text{ax}}\), respectively (Tsuno et al., 1986; Hescheler et al., 1987; Brown and Higashida, 1988; Shimahara et al., 1990; Taussig et al., 1992; Wilk-Blaszczak et al., 1994b). G\(_{\text{ax}}\) produces a fast inhibition, presumably by direct action on the channel (Hille, 1994), whereas G\(_{13}\) inhibits \(I_{\text{Ca,V}}\) slowly, possibly because of a multistep pathway (Wilk-Blaszczak et al., 1994b). Three members of the Rho family of monomeric G-proteins (Hall, 1994; Nobes and Hall, 1994; Mackay et al., 1995; Vojtek and Cooper, 1995; Ridley, 1996), Rac1, Cdc42, and RhoA, have been reported previously to be activated by G\(_{13}\) (Buhl et al., 1995; Collins et al., 1996; Hooley et al., 1996). Here, we present...
Rho family proteins are involved in the inhibition of \( I_{\text{Ca,V}} \) by BK. \( A_1 \) and \( A_2 \) show responses to BK either in a cell after a 20 min perfusion with GST-tagged Rho-GDI (0.2 \( \mu \)M) or a cell perfused with GST alone (0.2 \( \mu \)M). The \( I_{\text{Ca,V}} \) was activated by a 100 msec test command to 0 mV applied every 10 sec from a holding potential of ~90 mV (sampling, 10 kHz). Leakage- and capacitance-subtracted current traces are displayed, showing \( I_{\text{Ca,V}} \) before (Con) and at the peak of action by BK (0.1 \( \mu \)M). The continuous line marks the zero current, and the inset outlines the experimental set-up for intracellular perfusion. \( B_1 \) and \( B_2 \), Cumulative distributions of the responses to BK (0.1 \( \mu \)M; \( B_1 \)) and corresponding response indices (BK) in these cells that have been perfused with Rho-GDI (solid circles) or control solution (open squares). The cumulative distributions were constructed as follows. For each cell, the response to BK was expressed as percentage inhibition of the \( I_{\text{Ca,V}} \) before transmitter, and this value (X) was plotted on the horizontal axis. For each X, the fraction of responses (% Responses > X) larger than a given X was calculated and used to build the cumulative distribution. For example, the cumulative distribution in \( B_1 \) shows that ~90% of the control cells had a response to BK greater than zero (open squares), whereas only ~50% of the Rho-GDI-treated cells had a response greater than zero (solid circles). The responses indices (\( B_2 \)) are proportional to the areas under the corresponding cumulative distribution.

The asterisk denotes \( p < 0.02 \), compared with control values, whereas numbers indicate how many experiments were performed for each group.

**Evidence indicating that Rac1, and possibly also Cdc42, serve as intermediaries in the inhibitory pathway between G13 and \( I_{\text{Ca,V}} \).**

**Materials and methods**

Cultures of NG108-15 cells (passages 15–28) were prepared as described (Hamprecht et al., 1985; Wilk-Blaszczak et al., 1994b). Ciprofloxacin (10 mg/l, Miles, Kankakee, IL) was added to newly thawed cells for 2 weeks in culture medium (Sigma, St. Louis, MO), except for nucleotides (Boehringer Mannheim, Indianapolis, IN). Transmitters (Peninsula, Belmont, CA) were used at 0.1 \( \mu \)M and pipetted directly into the bath as described previously (Wilk-Blaszczak et al., 1994b). We consistently observed that prolonged dialysis per se did not significantly depress the responses to BK and Leu-Enk.

**Intrapipette perfusion and transmitter application.**

The effectiveness of the intrapipette perfusion method for delivering reagents in the cells was assessed based on the work of Pusch and Neher (1988). We calculated the time course of loading the NG108-15 cells using the following equation:

\[
\tau = (0.6 \pm 0.1) \cdot R_a \cdot M^{1/3} \cdot t
\]

where \( \tau \) = seconds, \( R_a \) = MΩ access resistance of pipette, \( M \) = daltons of diffusing substance. In our experiments, \( R_a \) was 0.5–1 MΩ. Because this equation was derived for cells with a 7–8 \( \mu \)m radius, we corrected the values of \( \tau \) for cells with a radius of 20 \( \mu \)m. For antibodies, \( \tau \) ranged between 3 and 12 min. The transmitter responses were measured after 20 min of perfusion, which allowed the antibodies (the largest molecules used in the present work) to diffuse for at least a time corresponding to at least twice the estimated \( \tau \) for loading the cell. To eliminate the effect of variability among cells, we alternated experiments on cells perfused with active reagents with cells treated with control solutions. For calcium current measurements (\( I_{\text{Ca,V}} \)), the extracellular solution contained (in mM): 125 NaCl, 5.4 CsCl, 1.8 CaCl₂, 1 MgCl₂, 10 HEPES, 5 glucose, and 0.005 tetrodotoxin, pH 7.4 (with NaOH). The use of Ca²⁺ as a charge carrier accounts for the relatively fast rate of inactivation of \( I_{\text{Ca,V}} \) compared with experiments in which Ba²⁺ was used (see Taussig et al., 1992). The pipette solution included (in mM): 112 CsCl, 1 MgCl₂, 10 EGTA, 30 HEPES, 3 ATP, and 0.1 GTP, pH 7.3 (with CsOH). For the measurement of the voltage-independent potassium current activated by BK (\( I_{\text{BK}} \)) (Brown and Higashida, 1988), equimolar concentrations of KCl replaced CsCl in both extracellular and intracellular solutions, whereas EGTA was lowered to 0.1 mM. All chemicals were obtained from Sigma (St. Louis, MO), except for nucleotides (Boehringer Mannheim, Indianapolis, IN).

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for the duration of the experiment both the raw and the subtracted traces. Only cells with small and stable leakage were used for the experiment.

Analysis of voltage-clamp data. The responses to BK were not normally distributed and were displayed as cumulative distributions of groups of data (McGehee et al., 1992; Wilk-Blaszczak et al., 1994), a method that provides direct information about the scattering of the measurements. For the purpose of immediate comparison, response indices (equal to the areas under each distribution) were used. We observed, much like findings in our previous work on the G_\text{I}-\text{Ca,V} pathway (Wilk-Blaszczak et al., 1994b), that agents that block this pathway (such as anti-G-protein antibodies) act in part by increasing the number of cells that do not respond to BK. For statistical purposes, we also counted as nonresponders cells with an occasional “run-up” coincident with the application of BK. We used the Fisher Exact Probability Test (one-tailed) for the comparison between groups of data. Responses to Leu-Enk were presented as mean percentage inhibitions ± SDs.

Biochemistry and molecular biology. Immunoblot analysis was performed as described previously (Wilk-Blaszczak et al., 1994b). Affinity-purified polyclonal antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), except for the anti-Rac2 (directed against Rac2 with an N-terminal hexahistidine tag). The specificity of anti Rac1, Cdc42, and Rho-A antibodies was validated by their ability to recognize the appropriate protein and by lack of cross-reactivity with other proteins. In the electrophysiological experiments, the antibodies were used at the final concentration of 0.8 μM.

The mRNA from NG108-15 cells was obtained using the guanidinium thiocyanate procedure, followed by oligo dT cellulose chromatography. For the RT-PCR technique, primers from the sequence of murine Rac1 were used (Moll et al., 1991). The sense primer was CAGTGAATCT-CCCTGCGGGTAG (574–554). The 287 bp product was subcloned using the TA cloning method, and its sequence was determined by double-stranded sequencing.

Recombinant Rho-GDI was expressed in Escherichia coli as a glutathione S-transferase (GST) fusion protein, and recombinant GST was produced in Spodoptera frugiperda cells with a baculovirus expression system. Both proteins were purified by chromatography through glutathione-Sepharose using standard procedures, dialyzed into the pipette solution (see above) without EGTA and stored in concentrated aliquots at −80°C. Each aliquot was diluted 10-fold with the pipette solution before use.

Peptides were used as sequences (see Fig. 3A, inset) near the C termini of Rac1 (a.a. 178–188), Rac2 (a.a. 178–188), Cdc42 (a.a. 178–188), and RhoA (a.a. 180–190), and were synthesized and purified (>95%) by Bio-Synthesis (Lewisville, TX). In the experiments using perfusion of anti-Rac1, anti-Cdc42, and anti-RhoA antibodies, the cells were treated with azide-free antibodies (0.8 μM, Santa Cruz Biotechnology) for 20 min.

RESULTS

Rho-GDI attenuates the response to BK

To determine whether Rho family proteins are involved in the responses to BK and Leu-Enk, we applied recombinant Rho-GDI intracellularly. Rho-GDI selectively binds to and inhibits guanine nucleotide exchange on monomeric G-proteins of the Rho family (Boguski and McCormick, 1993), thereby blocking interactions with downstream effectors. At the end of the application, the inhibition of I_{\text{Ca,V}} by BK was examined first. The inhibition by Leu-Enk was measured thereafter as a control. After application of Rho-GDI, the inhibition of I_{\text{Ca,V}} produced by BK was attenuated (Fig. 1A1, A2). A summary of the effects of Rho-GDI on the response to BK is presented in Figure 1B, which shows cumulative distributions (B1) and response indices (B2). In contrast, after application of Rho-GDI, inhibition of I_{\text{Ca,V}} produced by Leu-Enk was retained (cells treated with Rho-GDI, n = 20, 27 ± 18%; control cells, n = 14, 22 ± 12%). The blocking action of Rho-GDI on the response to BK was incomplete; one explanation is that availability of endogenous Rho-GDI is already high, as evinced by immunodetection. Incomplete inhibitory effects of excess Rho-GDI were observed previously (Lamaze et al., 1996).

In some experiments (n = 14), GST-Rho-GDI was used. In other experiments (n = 7), Rho-GDI was used after proteolytically cleaving GST-Rho-GDI with thrombin and removing the free GST by glutathione-Sepharose chromatography. The two preparations provided identical results. In control experiments, pipette solution was applied alone intracellularly (n = 8) or together with GST (n = 7). No significant direct effects of these proteins on peak I_{\text{Ca,V}} or holding current were observed. This effect of Rho-GDI suggests that a member of the Rho family of small G-proteins is involved in the inhibition of I_{\text{Ca,V}} by BK.

Rho family members are expressed in NG108-15 cells

Immunoblot analysis and RT-PCR were used to determine which members of the Rho family of small G-proteins are expressed in NG108-15 cells. Polyclonal antibodies against RhoA and Cdc42 detected proteins of the expected size in fractions of both membranes and cytosol from NG108-15 cells (Fig. 2). In contrast, polyclonal antibodies against Rac1 and Rac2, two monomeric G-proteins closely related to each other and to Cdc42, failed to identify appropriate polypeptides in NG108-15, although they robustly recognized the appropriate recombinant standard (data not shown). Because Rac2 is specific for myeloid cells, its detection was not expected in NG108-15 cells. However, Rac1 is expressed ubiquitously (Hall, 1994; Nobes and Hall, 1994; Mackay et al., 1995; Vojtek and Cooper, 1995; Ridley, 1996). To test
inhibition of $I_{\text{Ca,V}}$ by BK was blocked (Fig. 3A$_1$, A$_2$). In contrast, application of the Rac2 or RhoA peptides, as well as control pipette solution, under identical conditions, did not depress the response to BK. Importantly, none of the peptides significantly affected the response to Leu-Enk, measured in each cell after the response to BK (Rac1 peptide treatment, $n = 9$ cells, 27 ± 18% inhibition; Cdc42 peptide, $n = 9, 39$ ± 18%; RhoA peptide, $n = 15, 21$ ± 7%; Rac2 peptide, $n = 12, 31$ ± 14%; pipette solution, $n = 16, 32$ ± 16%). These findings suggest that Rac1 and the closely related Cdc42 are potentially involved in the inhibition of $I_{\text{Ca,V}}$ by BK. None of the peptides used produced direct inhibitory effects on peak $I_{\text{Ca,V}}$ or holding current.

### The anti-Rac1 antibody blocks the response to BK

To further test whether Rac1 and Cdc42 are involved in the response to BK, we intracellularly applied polyclonal antibodies directed against the C-terminal regions of each of these proteins. Previously, anti-Rac1, anti-Rac2, and anti-Cdc42 blocking antibodies identical to those used in these studies were used to demonstrate the involvement of Rac2 in the regulation of oxygen radical production in phagocytes (Knaus et al., 1991). These same antibodies recognized appropriate recombinant proteins in immunoblot analysis (see above). Using the intracellular perfusion method, we have found that the application of an anti-Rac1 antibody blocked the response to BK (Fig. 3B$_1$, B$_2$). If the same antibody was preincubated with the antigenic peptide, the response to BK was retained (Fig. 3B$_1$, B$_2$). The antigenic peptide alone did not affect the response to BK (8 μM) (Fig. 3B$_1$, B$_2$). In contrast to the action of the anti-Rac1 antibody, anti-Cdc42 and anti-RhoA antibodies did not block inhibition of $I_{\text{Ca,V}}$ by BK (Fig. 3B$_1$, B$_2$), although they recognized appropriate recombinant standards by immunoblot analysis (Fig. 2). We have also found that none of the treatments with antibodies significantly affected the response to Leu-Enk, measured in each cell after the response to BK (Rac1 antibody treatment, $n = 6$ cells, 25 ± 7% inhibition; peptide-blocked Rac1 antibody, $n = 4, 18$ ± 5%; Cdc42 antibody, $n = 9, 36$ ± 13%; RhoA antibody, $n = 4, 30$ ± 12%; Rac2 peptide alone, $n = 3, 41$ ± 4%; pipette solution, $n = 8, 29$ ± 11%).

BK and Leu-Enk both inhibit the same component of $I_{\text{Ca,V}}$ (Tausig et al., 1992; Wilk-Blaszczak et al., 1994b), but only the response to BK is blocked by the anti-Rac1 antibody. Therefore, it is unlikely that the effect of the antibody is attributable to direct suppression of $I_{\text{Ca,V}}$. We confirmed this idea by studying the effect of anti-Rac1 antibody on peak $I_{\text{Ca,V}}$ over a broad range of membrane potentials (Fig. 4A). As a control, current–voltage curves were obtained after perfusion of pipette solution. In cells treated with antibody (Fig. 4A$_1$, n = 2), the current–voltage relation was not altered significantly compared with one control cell (Fig. 4A$_1$), whereas the inhibition by BK was suppressed over the entire range of membrane potentials examined. Finally, the lack of any direct action of the anti-Rac1 antibody on $I_{\text{Ca,V}}$ was confirmed further by monitoring the peak $I_{\text{Ca,V}}$ for the duration of the experiment (Fig. 4B) (n = 7).

The specificity of the anti-Rac1 antibody for the $G_{13}$ pathway was tested additionally by examining whether this antibody blocks a second response to BK, activation of $I_{K,\text{BK}}$ (Brown and Higashida, 1988). This response is mediated by a distinct heterotrimeric G-protein, $G_{13/11}$ (Wilk-Blaszczak et al., 1994a). $I_{K,\text{BK}}$ was measured in cells perfused with either antibody or pipette solution (Fig. 5A$_1$, A$_2$). We observed that the anti-Rac1 antibody did not significantly reduce the activation of $I_{K,\text{BK}}$ by BK (Fig. 5B$_1$, B$_2$).

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**Figure 3.** Rac1 and/or Cdc42 mediate inhibition of $I_{\text{Ca,V}}$ by BK. $A_1$, $A_2$. Summary of the responses to BK (0.1 μM) after intracellular perfusion with the indicated C-terminal peptides (250 μM) or with pipette solution. The inset on the right displays the sequences of the peptides used. The cumulative distributions are shown in $A_1$, whereas $A_2$ displays the corresponding response indices. $B_1$, $B_2$. Summary of the responses to BK (0.1 μM) after intracellular perfusion with the indicated antibodies (0.8 μM; AB) or with Rac1 antigenic peptide (8 μM; solid triangles) or pipette solution (open squares). Rac1 antigenic peptide was incubated on ice with anti-Rac1 antibody for at least 30 min before perfusion (open circles). Other explanations are as in Figure 1B.

whether Rac1 is expressed in NG108-15 cells, we used the RT-PCR technique. Primers to the nonconserved regions of the mouse sequence were chosen to amplify the specific cDNA target sequence, generating a single product of the appropriate size (Fig. 2). The sequence obtained was identical to that of murine Rac1. The lack of detection of Rac1 by immunoblot analysis combined with positive detection by PCR suggests that Rac1 is a protein of low abundance (see also Stromstedt et al., 1994; Du et al., 1996; Tian et al., 1996). In conclusion, the evidence of expression of Rac1, Cdc42, and RhoA, along with the observed action of Rho-GDI, suggested that these monomeric G-proteins may be involved in the calcium current-inhibitory pathway of BK.

**C-terminal peptides from Rho family members block the response to BK**

To determine whether any of these monomeric G-proteins are involved in the inhibitory response to BK, we intracellularly applied synthetic peptides corresponding to the C-terminal region of Rac1, Rac2, Cdc42, and RhoA (Yeramian et al., 1987; Didsbury et al., 1989; Shinjo et al., 1990). Amino acid sequence homology among the Rho family proteins is least conserved within their C-terminal regions, which are thought to play key roles in interactions with effector and/or regulatory proteins. Based on these properties, synthetic peptides corresponding to Rac1 and Rac2 were used previously to demonstrate that Rac1 participates in activation and assembly of respiratory burst oxidase (Kreck et al., 1994). After application of either Rac1 or Cdc42 peptides, the
DISCUSSION

We have presented converging lines of evidence showing that activation of the monomeric G-protein Rac1 is required for the inhibitory action of BK on $I_{Ca,V}$ (Fig. 5C). This conclusion is supported by the observation that Rac1 is expressed in NG108-15 cells and that intracellularly applied anti-Rac1 antibody blocks the response to BK, and it is consistent with the effects of intracellularly perfused Rho-GDI and Rac1 peptide on the same response. We have studied in detail the action of the anti-Rac1 antibody on the inhibitory pathway of BK on $I_{Ca,V}$ and have observed that (1) the antibody effect is blocked by preincubation with the antigenic peptide, (2) it is not reproduced by any other antibodies used, and (3) it does not extend to the activation of $I_{Ca,V}$ by Leu-Enk. Low levels of expression of Rac1, as evinced by immunoblot analysis, might have rendered the blocking action of the perfused antibody more effective.

The involvement of Cdc42 in the inhibitory pathway of BK is not clear, because the inhibition of $I_{Ca,V}$ by BK is blocked by the Cdc42 peptide, but not by the anti-Cdc42 antibody. It is possible that the antibody might not have achieved during perfusion a sufficient concentration in the cells to block the response of Cdc42, which appears to be more abundant than Rac1 in NG108-15 cells. If both Rac1 and Cdc42 are required for the response to BK, they may operate in a cascade, as shown previously in other systems (Chant and Stowers, 1995). Alternatively, Cdc42 might not contribute to the response to BK in NG108-15 cells, and the blocking effect of the Cdc42 peptide on this response could be explained by its close sequence homology with the Rac1 peptide.

Although RhoA can couple to G13 (Buhl et al., 1995; Hooley et al., 1996) and is readily immunodetected in NG108-15 cells, the lack of effects of both the RhoA peptide and the anti-RhoA antibody makes it unlikely that this protein plays a role in the inhibition of $I_{Ca,V}$ by BK. Consistent with this idea, BK (in contrast to thrombin or lysophosphatidic acid) does not induce RhoA-dependent neurite retraction in NG108-15 cells (Moolekar, 1995).

Monomeric G-proteins of the Rho family are involved in a broad range of cellular functions, including regulation of cell shape and motility, vesicle trafficking, and transmembrane transport (Hall, 1994; Nobes and Hall, 1994; Buhl et al., 1995; Mackay et al., 1995; Ridley, 1995, 1996; Vojtek and Cooper, 1995; Collins et al., 1996; Hooley et al., 1996; Lamaze et al., 1996; Machesky and Hall, 1996). The present work demonstrates that Rac1 and possibly Cdc42 are required for the inhibition of neuronal $I_{Ca,V}$ by BK and, as a consequence, for regulation of excitability. Although the observed inhibitory actions of the G13-Rac1 pathway on the amplitude of $I_{Ca,V}$ are on average of moderate size, at presynaptic terminals they could have important physiological effects (attributable to the nonlinearity of the Ca$^{2+}$-dependency of transmitter release) (see Kandel et al., 1991). Furthermore, by allowing higher frequencies of action potential generation in primary sensory neurons (see Bleakman et al., 1990), inhibition of $I_{Ca,V}$ may enhance the BK-induced pain sensation (Dray and Perkins, 1993).

Our results parallel those recently obtained in muscles of Drosophila larvae (Zhong, 1995), where Ras was shown to mediate synaptic responses to a neuropeptide. Interestingly, Ras may mediate facilitation of $I_{Ca,V}$ by unknown receptors in neuronal cells (Collin et al., 1990; Hescheler et al., 1991; Hahnle et al., 1992; Fitzgerald and...
Dolphin, 1995), not inhibition as with Rac1 and Cdc42 (present work). It will be of interest to test the hypothesis that Rac1/Cdc42 inhibits $I_{Ca,V}$ via activation of specific MAPK pathways (Coso et al., 1995; Minden et al., 1995; Prasad et al., 1995; Zhang et al., 1995), as well as to examine the generality of these mechanisms in the inhibition of $I_{Ca,V}$ by neurotransmitters.

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