Regulation of Bradykinin- and ATP-activated Ca^{2+} -permeable Channels in Rat Pheochromocytoma (PC12) Cells

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Membrane currents activated by bradykinin (500 nM) and by extracellular ATP (50 μM) were studied in voltage-clamped, NGF-treated rat pheochromocytoma (PC12) cells. Under quasiphysiological ionic conditions, both substances caused an outward current due to opening of Ca^{2+} -activated K^+ channels. Bradykinin caused an additional inward current that could be studied after blockade by internal Cs^+ of the initial transient outward current. The inward current became larger when the extracellular Ca^{2+} concentration was increased. Neither inositol-1,4,5-trisphosphate, dioctanoylglycerol, phorbol 12-myristat 13-acetate, forskolin, GTP, $\text{GTP-}\gamma\text{-S}$, or pretreatment with pertussis toxin affected this current component. Increasing the internal Ca buffer concentration [EGTA or bis-(*o*-aminophenoxy)-ethane-*N,N,N',N'*-tetra-acetic acid] from 1 to 10 mM had no effect on the inward current as long as the free $[\text{Ca}^{2+}]_i$ was kept constant. However, it was modulated by the resting free $[\text{Ca}^{2+}]_i$. Elevation of $[\text{Ca}^{2+}]_i$ from nominally 0 to 60 or to 180 nM increased the bradykinin-induced average peak current density from 0.14 to 1.04 or to 2.29 pA/pF, respectively. This regulation may depend on a calmodulin-dependent pathway, since CGS 9343B, a calmodulin inhibitor, blocked the effect of elevated $[\text{Ca}^{2+}]_i$. With ATP as an agonist, outward current was preceded by a large inward current that was partially blocked by extracellular Ca^{2+} in the millimolar range. Extracellular Ca^{2+} was also found to reduce the single-channel conductance estimated from outside-out patches treated with ATP.

Several neurotransmitters act upon their target cells by raising the free intracellular calcium concentration, $[\text{Ca}^{2+}]_i$. This also holds true for bradykinin, which has been reported to induce Ca^{2+} release from internal stores via inositol-1,4,5-trisphosphate (IP_3 ; Fasolato et al., 1988; Thayer et al., 1988b; Ogura et al., 1990). Besides this effect on Ca^{2+} release from intracellular stores, bradykinin opens a pathway for external Ca^{2+} to enter the cell (Schilling et al., 1989; Fasolato et al., 1990; Weiss et al., 1990; Buchan and Martin, 1991). Both effects are mediated by B_2 -type receptors that are present in PC12 cells (Fasolato et al., 1988). Whereas the mechanism leading to intracellular Ca^{2+} release is rather well understood, relatively little is known about the activation of Ca^{2+} entry from outside the cell. In some prep-

arations (e.g., dorsal root ganglia of the rat; Burgess et al., 1989), voltage-activated Ca^{2+} channels seem to be involved, which open upon cell depolarization due to an Na^+ inward current induced by bradykinin. However, in PC12 cells several lines of evidence, including electrophysiological studies and experiments with Ca^{2+} channel blockers, suggest another type of Ca^{2+} -permeable ion channel to be opened by bradykinin (Fasolato et al., 1988; Weiss et al., 1990). Undifferentiated PC12 cells also respond to ATP with an increase in $[\text{Ca}^{2+}]_i$. The receptors for ATP in this cell line have been reported to be P_2 purinergic (Nakazawa et al., 1990a). As in the case of bradykinin, there is strong evidence that the rise in $[\text{Ca}^{2+}]_i$ is at least partially due to an influx of Ca^{2+} from outside the cell (Fasolato et al., 1990).

The aim of this article is to compare the action of bradykinin and ATP on membrane currents in PC12 cells differentiated by 3–7 d exposure to NGF. Particular attention was focused on the role of external Ca^{2+} as possible charge carrier for the inward currents evoked by both substances. The mechanism by which the bradykinin-induced inward current is regulated is still unknown. However, we demonstrate its modulation by $[\text{Ca}^{2+}]_i$, probably through a calmodulin-dependent reaction. We provide evidence that bradykinin and ATP open separate classes of Ca^{2+} -permeable channels.

A preliminary account of this work has been published (Neuhaus et al., 1991).

Materials and Methods

Cell culture. All experiments were performed with rat pheochromocytoma (PC12) cells from a clone kindly provided by Dr. U. Otten (Department of Physiology, University of Basel, Switzerland). This cell line shows differentiation within 2–3 d when exposed to NGF. Cells were grown in Dulbecco's modified Eagle's medium with 10% horse serum (GIBCO) in a 5% CO_2 atmosphere at 37°C. They were plated on poly-L-lysine-coated dishes at a density of 5×10^3 cells ml^{-1} . Differentiation was induced by addition of 100 ng ml^{-1} NGF (7S fraction, Sigma N-0513) and by reducing the horse serum concentration to 5% (Usovich et al., 1990).

Solutions. The standard external solution was a modified Tyrode's solution consisting of (in mM) 150 NaCl, 5 KCl, 1.8 CaCl_2 , 1 MgCl_2 , and 10 HEPES, with pH adjusted to 7.4 with NaOH. The CaCl_2 concentration of this solution was varied as indicated in the text. In Na^+ -free solutions, NaCl was replaced by choline Cl. In another set of experiments, a Cl^- -free external solution was used containing (in mM) 135 Na-aspartate, 5 K-aspartate, 1 Mg-aspartate, 10 Ca-aspartate, and 10 HEPES, with pH adjusted to 7.4 with NaOH. Pipettes were filled with one of three internal solutions: a "KCl solution" consisting of (in mM) 135 KCl, 1 MgCl_2 , 0.5 CaCl_2 , 1 EGTA, 1 Na_2ATP , and 10 HEPES, with pH adjusted to 7.4 with NaOH; a "CsCl solution" in which KCl was replaced by CsCl in order to block voltage-dependent K channels; or an "aspartate solution," in which Cl^- was replaced by aspartate. The free Ca^{2+} concentration in these solutions was calculated to be 60 nM, which is close to empirical values obtained from measurements with fura-2 in rat sympathetic neurons (Thayer et al., 1988b) and in differ-

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entiated PC12 cells (Reber and Reuter, 1991). Where indicated, $[Ca^{2+}]_i$ was changed to nominally 0 or 180 nM. Calculations were performed with a program written by Dr. J. Kentish, University College London, based on the apparent dissociation constants published by Fabiato and Fabiato (1979). In some experiments, the Ca^{2+} buffer capacity was increased using 10 mM bis-(*o*-aminophenoxy)-ethane-*N,N,N',N'*-tetraacetic acid (BAPTA). Binding constants for BAPTA were taken from a publication by Tsien (1980). Bradykinin- and ATP-containing solutions were freshly prepared from aqueous stock solutions (50 μ M bradykinin, 200 mM Na_2ATP) kept at $-25^\circ C$. The calmodulin inhibitor CGS 9343B was dissolved in dimethyl sulfoxide (DMSO) and stored at $-25^\circ C$. The final concentration of DMSO in the experimental solutions was 0.1%. Caffeine-containing solutions were freshly prepared from powder. Suramin was a generous gift from Bayer AG, Wuppertal, Germany; and CGS 9343B, from Ciba-Geigy AG, Basel, Switzerland. Substances were applied into the bathing solution by microperfusion through a capillary tube with an inner diameter of about 100 μ m. With this arrangement, a complete exchange of the solution around a cell was achieved within 2.1 ± 0.3 sec ($n = 8$). This value was obtained from voltage-clamped cells held at -30 mV by measuring the current response to a change from a control solution (5 mM K^+) to one containing 30 mM K^+ . All experiments were done at room temperature (20 – $25^\circ C$).

Electrical recording and data analysis. Membrane currents were recorded from voltage-clamped PC12 cells with patch pipettes (Hamill et al., 1981) using a List EPC-7 patch-clamp amplifier. Pipettes (borosilicate glass, Clark Electromedical Instruments) had resistances of 2–5 M Ω for whole-cell patches and about 10 M Ω for excised patches. Single-channel currents from outside-out patches and whole-cell currents obtained with voltage ramps were low-pass filtered (2 kHz, -3 dB) and digitized on line (10 kHz sample frequency). Currents from all other experiments were stored on an FM tape recorder (Racal 4DS) and low-pass filtered at 50 Hz (-3 dB) prior to digitization. Analysis was done with a microcomputer using the EPC program (Cambridge Electronic Design, Cambridge, United Kingdom) and a program written in our lab by Dr. A. B. Cachelin. If not stated otherwise, statistics are given as means $\pm$ SEM.

Results

Bradykinin-activated currents

Figure 1 compares whole-cell current responses to applications of 500 nM bradykinin, 10 mM caffeine, and 50 μ M ATP. Bradykinin and caffeine effects were similar in showing an initial outward current that depended on the release of intracellular Ca^{2+} (Brown and Higashida, 1988; Thayer et al., 1988a; Reber and Reuter, 1991). However, with bradykinin a second, inward current component could often be observed that was absent in the caffeine response. The ATP-activated current showed an initial inward component followed by an outward current that was seen only after several seconds. Outward currents depended on a rise in $[Ca^{2+}]_i$ since they could be suppressed by 10 mM EGTA or BAPTA in the pipette solution.

The inward current observed with bradykinin was investigated in detail with internal Cs^+ as blocker of K^+ channels. In some experiments a Cs^+ -resistant small transient outward current remained. With the standard external and internal ($CsCl$) solutions, the average peak current density of the inward current was 0.55 ± 0.05 pA/pF ($n = 8$) at -50 mV holding potential. This inward current is carried by cations and is not due to a Cl^- efflux. With aspartate-containing intracellular and extracellular solutions, the inward current was still present in 7 out of 10 cells. However, the bradykinin-induced inward current was strongly dependent on the extracellular Ca^{2+} concentration. The average peak current density increased from 0.35 ± 0.06 pA/pF ($n = 12$) in nominally Ca^{2+} -free medium to 1.31 ± 0.14 pA/pF ($n = 18$) with 10 mM external Ca^{2+} (Fig. 2). Inward currents could still be measured after replacement of NaCl by choline Cl in the Ca^{2+} -containing (10 mM) external medium. This indicates that Ca^{2+} is a main current carrier of the bradykinin-

Figure 1. Whole-cell membrane currents induced by 500 nM bradykinin (*A*), 10 mM caffeine (*B*), and 50 μ M ATP (*C*). Internal solution: KCl solution (see Materials and Methods). Holding potential, -50 mV. Broken lines indicate current levels before drug application. Note the different current scales.

activated inward current. The voltage dependence of the inward current was studied by applying voltage ramps ranging from $+50$ to -150 mV (Fig. 3). The current-voltage relation revealed a slight nonlinearity between 0 and -150 mV, with an increase in conductance at more negative potentials. The reversal potential (E_R) was near 0 mV in four experiments. Exact estimates were not possible because of the small current size near E_R and interference with voltage-activated Ca^{2+} channels not blocked by verapamil and ω -conotoxin. Although $CsCl$ instead of KCl was used in the internal medium, we cannot exclude a small Cs^+ current through Ca^{2+} -activated K^+ channels that influences the positive branch of the I/V curve. In this case, the true reversal potential for the bradykinin-induced inward current would be underestimated. The results suggest that bradykinin activates cation channels that pass Na^+ , Cs^+ , and Ca^{2+} , with a preference in selectivity for Ca^{2+} . We were unable to resolve single-channel events underlying this current component. The extra noise on the current records during full activation of the inward current by bradykinin exceeded the background current fluctuations at most by a factor of 2. It was too small to allow reliable fluctuation analysis of channel currents and kinetics.

Another set of experiments was performed to obtain information about the regulation of the pathway that leads to activation of the observed inward current. We could not find an

Figure 2. Dependence of bradykinin-activated (500 nM) inward currents on the extracellular Ca^{2+} concentration. Internal solution: CsCl solution (see Materials and Methods). Holding potential, -50 mV. *A*, Current traces from two cells without added Ca^{2+} (upper trace) and with 10 mM external Ca^{2+} (lower trace), respectively. Broken lines indicate current levels before drug application. *B*, Bradykinin-induced inward current densities (pA/pF) from a total of 38 cells with 10 mM, 1.8 mM, and no added external Ca^{2+} , right to left. Numbers above error bars indicate numbers of tested cells.

inhibition of the bradykinin-induced response after incubation of the cells with pertussis toxin (250 ng/ml, for 12–24 hr), suggesting that the G-proteins G_i and G_o are not involved. Furthermore, the inward current was not affected by intracellular application of IP_3 (50 μM), GTP (1 μM), or GTP- γ -S (100 μM), and by extracellular application of the protein kinase C activators dioctanoylglycerol (10 and 100 μM) and phorbol 12-myristate 13-acetate (1 and 10 μM), or of forskolin (10 μM), which activates protein kinase A. On the other hand, IP_3 in the pipette solution did produce an outward current due to Ca^{2+} release from intracellular stores. The inward current response was shown to be independent of transient Ca^{2+} release, since it was not reduced when internal Ca^{2+} buffer concentrations (EGTA or

Figure 3. *A*, Whole-cell currents recorded during a ramplike change of the membrane potential. The membrane potential was stepped from a holding potential of -50 mV to $+50$ mV and then ramped to -150 mV within 200 msec (top scale). The two current traces were recorded before and after application of 500 nM bradykinin. Internal solution, CsCl solution. External solution contained 10 mM Ca^{2+} , 0.5 μM TTX, 1 μM ω -conotoxin, and 10 μM verapamil. Note persistence of a small voltage-activated inward current. *B*, Bradykinin-induced current from the same experiment obtained by subtraction of the two current traces in *A*. The broken line indicates the zero-current level.

BAPTA) were increased to 10 mM, while the same treatment, but with internal KCl solution, caused a reduction of the outward current from 1.7 to 0.4 pA/pF. Moreover, caffeine (10 mM) produced a large outward but no inward current (Fig. 1). However, we found a strong dependence of the bradykinin-induced inward current on the steady state intracellular free Ca^{2+} concentration (Fig. 4). Without Ca^{2+} , but with 10 mM BAPTA in the pipette, the average peak current density was 0.14 ± 0.07 pA/pF ($n = 6$) compared to 1.04 ± 0.21 pA/pF ($n = 5$) when the internal solution contained 60 nM free Ca^{2+} , and 2.29 ± 0.30 pA/pF ($n = 6$) with 180 nM internal Ca^{2+} buffered with 10 mM BAPTA. This suggests that the free resting $[\text{Ca}^{2+}]_i$ is a regulating factor for the inward current response with bradykinin.

How does steady state $[\text{Ca}^{2+}]_i$ regulate the bradykinin-activated inward current? We used the calmodulin inhibitor CGS 9343B (Norman et al., 1987) and found that the effect of 180 nM $[\text{Ca}^{2+}]_i$ (buffered with 1 mM BAPTA) on this current component was greatly reduced in a dose-dependent manner (Fig. 5). This may suggest an involvement of a calmodulin-dependent

Figure 4. Dependence of bradykinin-activated (500 nM) inward current densities (pA/pF) on the intracellular free Ca^{2+} concentration. Holding potential, -50 mV. External solution with 10 mM Ca^{2+} ; internal solution, CsCl solution with 10 mM BAPTA and different free Ca^{2+} concentrations as indicated (for calculation of the free Ca^{2+} concentrations, see Materials and Methods). Numbers above error bars indicate number of measured cells.

pathway in the regulation of the bradykinin-induced current. One possible mechanism would be phosphorylation by a calmodulin-dependent kinase of the ion channels carrying this current, but other Ca^{2+} /calmodulin-dependent or -independent reactions have not been excluded.

ATP-activated currents

We studied currents activated by external ATP in our PC12 clone in order to find out whether they might be regulated by mechanisms comparable to those involved in bradykinin-induced currents.

The inward current activated by ATP (Fig. 6) was much faster in its onset than the bradykinin-induced inward current, with an average time to peak of 2.5 sec as compared to 25.7 sec with bradykinin. This value was very close to the time required for a complete exchange of solution by our perfusion system (2.1 sec; see Materials and Methods). External Mg^{2+} was not a necessary cofactor for the ATP response. As with bradykinin, the current was not inhibited by replacing Cl^- with aspartate in the external and internal solutions. Compared to bradykinin, the ATP-induced inward current was very pronounced but with large variations from cell to cell (974 ± 224 pA, $n = 7$, at -50 mV) independent of cell size, and it preceded a slowly rising outward current. The latter could be blocked when the internal solution was Ca^{2+} free and buffered with 10 mM EGTA (Fig. 6B), suggesting the involvement of Ca^{2+} -activated K^+ channels. The inward current was not affected by this treatment. Another point of interest was the effect of external Ca^{2+} on the ATP-induced inward current. This was studied by stimulating the same cell with ATP twice, in the presence of high and low

Figure 5. Dose-dependent effect of CGS 9343B on bradykinin-activated (500 nM) inward current densities (pA/pF). Holding potential, -50 mV. External solution with 10 mM Ca^{2+} ; internal solution, CsCl solution with 1 mM BAPTA and 180 nM free Ca^{2+} . CGS 9343B was applied to both external and internal solutions. Numbers above error bars indicate number of measured cells.

external Ca^{2+} concentrations, respectively. An example of such an experiment is shown in Figure 6A, in which the first current response to ATP was recorded with 10 mM external Ca^{2+} and, after a washout period, the second response with 1.8 mM $[\text{Ca}^{2+}]_o$. Similar results were obtained from three other cells. The inward current with 1.8 mM external Ca^{2+} was 1.5–3.5-fold larger than with 10 mM external Ca^{2+} . This is opposite to the effect seen with bradykinin-activated inward currents.

With ATP as an agonist, we were able to record single-channel activity from excised, outside-out patches (Fig. 7). Single-channel currents could be recorded at membrane potentials between -30 and -150 mV for up to 3 min after application of 20 μM ATP. No openings were detected at positive patch potentials. Despite this strong inward rectification at positive potentials, the current-voltage relation was almost linear in the range from -50 to -90 mV. Slope conductance estimates were therefore restricted to this range of membrane potentials (Fig. 7). In an external medium with 1.8 mM Ca^{2+} , the slope conductance was 17.2 ± 1.0 pS ($n = 3$). The inhibitory effect of external Ca^{2+} could also be shown at the single-channel level. With 10 mM external Ca^{2+} , single-channel amplitudes were decreased and the slope conductance was reduced to 9.1 ± 0.8 pS ($n = 4$). By contrast, the conductance increased to 26.5 ± 1.7 pS ($n = 3$) when Ca^{2+} was omitted from the bath solution (no added CaCl_2). The reduction of single-channel current amplitudes with external Ca^{2+} was not due to an open channel blockade, since we could not detect an increase in brief closures when measurements were done at high-frequency resolution ($f_c = 10$ kHz).

As found by others (Nakazawa et al., 1990b), suramin (50 μM) inhibited the ATP-activated inward current, while the bradykinin-activated inward current remained unaffected by this drug (data not shown).

Figure 6. Whole-cell membrane currents activated by 50 μM ATP. Holding potential, -50 mV. *A*, Current traces from one cell exposed to external solutions with 10 and 1.8 mM Ca^{2+} as indicated. The solution with 10 mM Ca^{2+} was tested first. Internal solution, CsCl solution. *B*, ATP responses from two different cells with KCl solution in the pipette. The lower trace was obtained with 10 mM EGTA and no added CaCl_2 in the internal solution. The broken line indicates the current level at -50 mV before application of ATP.

Discussion

In the present article, we compared the effects of bradykinin and ATP on membrane currents in differentiated PC12 cells. Bradykinin is a nonapeptide that is released in many tissues of the body, particularly after injury. It stimulates pain, causes vasodilatation, and releases neurotransmitters from neuronal cells (for review, see Miller, 1987). The cellular reactions induced by bradykinin have been the subject of intense studies. Among others, the peptide activates phospholipases C and A_2 , thus increasing second messenger molecules such as IP_3 , diacylglycerol, or arachidonic acid (Fasolato et al., 1988; Thayer et

Figure 7. Single-channel current measurements from excised outside-out patches exposed to 20 μM ATP. *A*, Single-channel traces from two patches clamped at various membrane potentials as indicated. The external solution contained no added Ca^{2+} (left) or 10 mM external Ca^{2+} (right), respectively. Internal solution, CsCl solution. *B*, Single-channel current-voltage relations obtained from the two patches shown in *A* and from another patch under similar conditions but with 1.8 mM external Ca^{2+} . Lines indicate linear regressions that were calculated from the current amplitudes between -50 and -90 mV. Error bars denote SD.

al., 1988b; Burgess et al., 1989). In addition, bradykinin activates a Ca^{2+} -dependent K^+ current that is probably the result of an IP_3 -induced Ca^{2+} release (Schmid-Antomarchi et al., 1986; Brown and Higashida, 1988). The peptide also activates an inward current in PC12 cells and sensory neurons. The ionic nature of this current has not yet been clearly identified in PC12 cells (Fasolato et al., 1988) and seems to be due to an influx of Na^+ ions in sensory neurons (Burgess et al., 1989). On the other hand, it has been shown by means of ^{45}Ca flux measurements and by fura-2 measurements that bradykinin also induces a Ca^{2+}

influx (Fasolato et al., 1988; Burgess et al., 1989; Schilling et al., 1989). In PC12 cells, this Ca^{2+} influx is thought to be activated independently of voltage-gated channels and to be involved in neurotransmitter release (Appell and Barefoot, 1989; Weiss et al., 1990).

Inward currents activated by extracellular ATP have been described for a variety of excitable cells, and the respective channel properties seem to be similar but not identical in different cell types (for review, see Bean and Friel, 1990). The reversal potential for the current is near 0 mV, indicating a rather nonselective cation conductance. No second messenger that modulates channel activity has so far been identified (Bean and Friel, 1990). The reason for us to compare the properties of bradykinin- and ATP-activated currents in PC12 cells was the possibility that bradykinin releases ATP together with neurotransmitters. ATP, in turn, could activate cation channels similar or identical to those opened by bradykinin. This is obviously not the case. The two conductance pathways are very different.

The most interesting and novel observation in our study concerns the bradykinin-induced inward current. We could show that this current strongly depends on extracellular and intracellular Ca^{2+} concentrations. While the dependence on extracellular Ca^{2+} is most easily explained by assuming that this ion is a major charge carrier, intracellular Ca^{2+} has an important modulatory effect. Intracellular Ca^{2+} by itself does not induce this conductance. Bradykinin binding to B_2 -receptors (Fasolato et al., 1988) is an essential condition for activation of the inward current. Several investigators have suggested that in PC12 cells a conductance pathway for Ca^{2+} is opened by bradykinin. However, the evidence for this has been rather indirect, either on the basis of membrane depolarizations recorded with voltage-sensitive dyes and simultaneous measurements of $[\text{Ca}^{2+}]$, (Fasolato et al., 1990), or inferred from divalent cation dependence of bradykinin-induced noradrenaline release (Weiss et al., 1990). Our experiments confirm these notions by direct analysis of the Ca^{2+} dependence of the bradykinin-induced inward current. Our results suggest a preference in selectivity of the presumptive channels for Ca^{2+} over Na^+ and Cs^+ . Small, agonist-activated Ca^{2+} currents resembling the one we have observed have been reported to be present in rat peritoneal mast cells (Matthews et al., 1989) and human leukemic T cells (Lewis and Cahalan, 1989). These currents are also slow and voltage insensitive and develop without a measurable increase in current fluctuations. However, a regulation by $[\text{Ca}^{2+}]$, as in our PC12 cells has not been reported for the other cell types. On the other hand, a similar dependence on resting $[\text{Ca}^{2+}]$, has recently been shown for the muscarinic suppression of M-currents in rat sympathetic neurons (Beech et al., 1991). Our experiments suggest a Ca^{2+} /calmodulin-dependent reaction that regulates the bradykinin-induced inward current. This interpretation rests on the high sensitivity of the current to changes in $[\text{Ca}^{2+}]$, and on the inhibitory effect of CGS 9343B, a recently described inhibitor of calmodulin activity (Norman et al., 1987; Polak et al., 1991). The half-maximal inhibitory concentration (between 3 and 10 μM) in our experiments is close to the K_m for inhibition of calmodulin-stimulated cAMP phosphodiesterase activity (3.3 μM ; Norman et al., 1987). It will be interesting to see how calmodulin regulates the bradykinin-activated inward current.

Our results on ion channels activated by extracellular ATP are in good agreement with those recently reported by Nakazawa et al. (1990a,b) for PC12 cells. The inhibitory effect of Ca^{2+} on

these channels resembles that on the Na^+ conductance of L-type Ca^{2+} channels (Tsien et al., 1987), although the potency is almost 3 orders of magnitude smaller. Unpublished observations (B. F. X. Reber, R. Neuhaus, and H. Reuter) with fura-2 strongly suggest that Ca^{2+} permeates through these channels, a conclusion also reached by Nakazawa et al. (1990a). The fact that ATP activates the channels in excised outside-out patches suggests a direct coupling between ATP binding to its purinergic receptor and channel opening, without involvement of a diffusible second messenger.

Our electrophysiological analysis of the bradykinin- and ATP-activated conductance excludes a common pathway. A similar conclusion, based on more indirect evidence, has recently been reached by Fasolato et al. (1990).

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