ELSEVIER

European Journal of Pharmacology 350 (1998) 293–299

ejp

Effects of cytosolic ATP and other nucleotides on Ca^{2+}-activated K^+ channels in cultured bovine adrenal chromaffin cells

Chunhe Chen [a], Hitoshi Houchi [b], Toshiaki Tamaki [b], Yutaka Nakaya [a,*]

[a] *Department of Nutrition, School of Medicine, University of Tokushima, Tokushima 770, Japan*
[b] *Department of Pharmacology, School of Medicine, University of Tokushima, Tokushima 770, Japan*

Received 30 December 1997; revised 17 March 1998; accepted 19 March 1998

Abstract

The effects of cytosolic ATP on Ca^{2+}-dependent K^+ (K_{Ca}) channel activation in cultured bovine adrenal chromaffin cells were investigated by using single-channel recording patch-clamp techniques. Application of ATP to the intracellular surface of excised inside-out patches activated K_{Ca} channels in a dose-dependent manner at 30 μM to 10 mM. The K_{Ca} channels also were activated by 3 mM of adenosine 5′-*O*-(3′-thiotriphosphate) (ATPγS), a non-hydrolyzable analogue of ATP, but not by 5′-adenylylimidodiphosphate (AMP-PNP) (from 300 μM to 3 mM). Furthermore, other nucleotides also activated K_{Ca} channels in inside-out patches. This modulation took place without addition of exogenous protein kinase and was dependent on the presence of Mg^{2+} in the bathing solution. Staurosporine, a non-specific kinase inhibitor, or H-89 (*N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinoline-sulfonamide), a cAMP-dependent protein kinase inhibitor, was unable to alter ATP-mediated K_{Ca} channel activation. Following complete removal of Mg^{2+}, a higher concentration of ATP (10 mM) and other nucleotides was required to activate K_{Ca} channels; however, Mg^{2+} was ineffective in altering the activation of K_{Ca} channels by itself. It is concluded that intracellular ATP and other nucleotides activate K_{Ca} channels directly. These nucleotides may regulate catecholamine release by changing the cell membrane potential in adrenal chromaffin cells.

Keywords: K^+ channel; Chromaffin cell; Nucleotide; $[Ca^{2+}]_i$

1. Introduction

Chromaffin cells from the bovine adrenal medulla synthesize, store, and secrete a complex mixture of hormones, the most important of which is adrenaline. Chromaffin cells express neurotransmitter receptors and generate action potentials as part of excitation–secretion coupling. Adenosine triphosphate (ATP), an established neurotransmitter, causes elevation of cytosolic Ca^{2+} and catecholamine secretion when applied to chromaffin cells at low concentrations (Asano et al., 1995; Reichsman et al., 1995), but at high concentrations it inhibits catecholamine secretion by suppression of voltage-dependent Ca^{2+} channels (Gandia et al., 1993). In isolated bovine chromaffin cells, extracellular ATP also inhibits acetylcholine-stimulated secretion (Chern et al., 1987). However, there have been few studies on the effect of intracellular ATP.

The Ca^{2+}-dependent K^+ channel (K_{Ca} channel) whose activation is regulated by intracellular Ca^{2+} and membrane depolarization has been identified and characterized in chromaffin cells (Marty, 1981; Yellen, 1984; Marty and Neher, 1985; Artalejo et al., 1993). Although the precise role of these channels is not clear, they have been implicated in a number of important physiological processes such as the regulation of catecholamine secretion in adrenal chromaffin cells (Petersen and Maruyama, 1984; Marty and Neher, 1985; Uceda et al., 1992). In a series of recent papers, direct activation of K_{Ca} channels by ATP has been consistently reported in a number of tissues (Chung et al., 1991; Albarwani et al., 1994; Bielefeldt and Jackson, 1994; Esguerra et al., 1994; Lee et al., 1995; Hartley and Kozlowski, 1996). However, in the case of chromaffin cells the mechanisms responsible for the action of cytosolic ATP have not been fully elucidated. We hypothesized that ATP inhibits catecholamine secretion, at least in part, by activation of K_{Ca} channels. To investigate this hypothe-

* Corresponding author. Tel.: +81-886-33-7090; fax: +81-886-33-7113; e-mail: nakaya@nutr.med.tokushima-u.ac.jp

0014-2999/98/$19.00

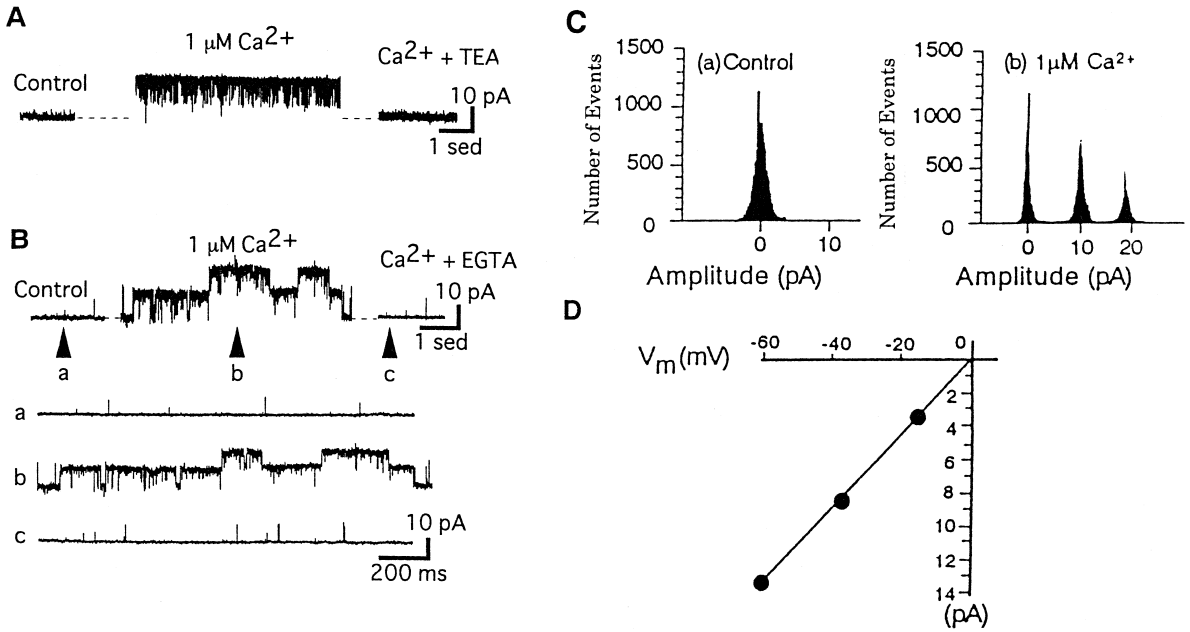

Fig. 1. Stimulatory regulation of the K_{Ca} channel by increasing internal Ca^{2+}. K_{Ca} channel currents were recorded under control conditions at +40 mV membrane potential (V_m) with 10^{-7} M internal Ca^{2+} concentration. Single-channel currents required 10^{-6} M concentration of internal Ca^{2+} for activity. (A) K_{Ca} channels were activated by 10^{-6} M Ca^{2+} applied to the cytosolic side and were blocked by 4 mM tetraethylammonium (TEA). (B) K_{Ca} currents were recorded under control conditions (trace a); K_{Ca} channels were activated by 10^{-6} M Ca^{2+} (trace b) and were suppressed by lowering cytosolic Ca^{2+} with 4 mM EGTA (trace c). The histogram and the I/V curve are given to illustrate the single channel in (C) and (D), respectively. The bath solution contained 100 mM K-aspartate, 40 mM KCl, 10 mM K-MOPS buffer, 1 mM Mg^{2+}, and 10^{-7} M Ca^{2+}, pH 7.2. The pipette solution contained 140 mM KCl, 10 mM K-MOPS, and 10^{-4} M Ca^{2+}, pH 7.2.

sis we used inside-out patch-clamp techniques to study this problem.

The results of the present study suggest that intracellular nucleotides directly modulate K_{Ca} channels in cultured bovine adrenal chromaffin cells. Furthermore, on the basis of our results when using staurosporine and H-89 (*N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide), it is unlikely that K_{Ca} channel activation in adrenal chromaffin cells is subject to modulation by the activity of the closely associated kinase.

2. Materials and methods

2.1. Single cell preparation

Isolated bovine adrenal chromaffin cells were dispersed enzymatically as described previously (Greenberg and Zinder, 1982). Briefly, the medulla was sliced with a hand slicer, and the slices were digested in medium containing 0.1% collagenase, 0.01% soybean trypsin inhibitor, and 0.5% bovine serum albumin in balanced salt solution (BSS) (135 mM NaCl, 5.6 mM KCl, 1.2 mM $MgSO_4$, 2.2 mM $CaCl_2$, 10 mM glucose, and 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)/NaOH; pH 7.40). Cells were plated in 35-mm culture dishes and were maintained for 3 to 6 days as monolayer cultures in Eagle's basal medium supplemented with 5% heat-inactivated fetal calf serum. Four or five glass coverslips were placed on the bottom of each culture dish.

2.2. Solutions and chemicals

High K^+ solution contained 100 mM K-aspartate, 40 mM KCl, 10 mM K-3-(*N*-morpholino)propane-sulfonic acid (MOPS) buffer, pH 7.2. Ca^{2+}-EGTA buffer was used to adjust the Ca^{2+} concentrations when they were less than 5×10^{-5} (mmol/l). Adenosine 5′-*O*-(3′-thiotriphosphate) (ATPγS), 5′-adenylylimidodiphosphate (AMP-PNP), guanylylimidodiphosphate (GMP-PNP), ATP, GTP, and ADP were purchased from Sigma (St. Louis, USA). GDP was purchased from Nakarai Tesque (Tokyo, Japan).

2.3. Electrophysiological recordings

Membrane currents were recorded in inside-out patches with a patch-clamp amplifier as previously described (Hamill et al., 1981). Soft glass pipettes prepared by an electrode puller (PP-83, Narishige, Tokyo, Japan) were used after being coated with Sylgard. The electrical resistance of the patch pipette was 3 to 5 MΩ for single channel recordings. Experiments were conducted at a temperature of 35 to 37°C. pClamp Version 6.0. (Axon Instruments, Foster City, CA) was used for data acquisition and analysis of single-channel currents. The open probability (NP_o) was determined from current amplitude histograms and was calculated using the following equation:

$$NP_o = \sum_{n=0}^{N} (nP_n)$$

where n is the number of channels in the patch and P_n is the integrated channel opening. Statistical comparisons were made by using Student's paired t-test, and results are presented as means ± S.E.M.

The length of recording for the NP_o calculations was more than 1 min.

3. Results

3.1. Characterization and identification of the K_{Ca} channel in chromaffin cells

The existence of K_{Ca} channels which have a single-channel conductance that ranges, depending on the recording conditions, from 96 to 300 pS and which are sensitive to the internal free Ca^{2+} concentration ($[Ca^{2+}]_i$) has been demonstrated by using the patch-clamp technique in cultured chromaffin cells (Marty, 1981; Yellen, 1984; Marty and Neher, 1985). In the present study, isolated KCa channels had a conductance of 215 ± 23 pS in symmetrical 150 mM KCl solution. When the pipette solution contained 1 nM charybdotoxin, ATP was still able to activate K_{Ca} channels. Moreover, charybdotoxin (1 nM) was ineffective against ATP-mediated channel activation at the internal side, indicating that this channel was charybdotoxin-insensitive ($n = 4$, data not shown).

Fig. 1A shows the effect of Ca^{2+} on single-channel currents in inside-out patches. Single-channel currents were observed infrequently under control conditions. The channels required micromolar concentrations of internal Ca^{2+} for activation and were blocked by 4 mM tetraethylammonium, a K^+ channel inhibitor. These channels also

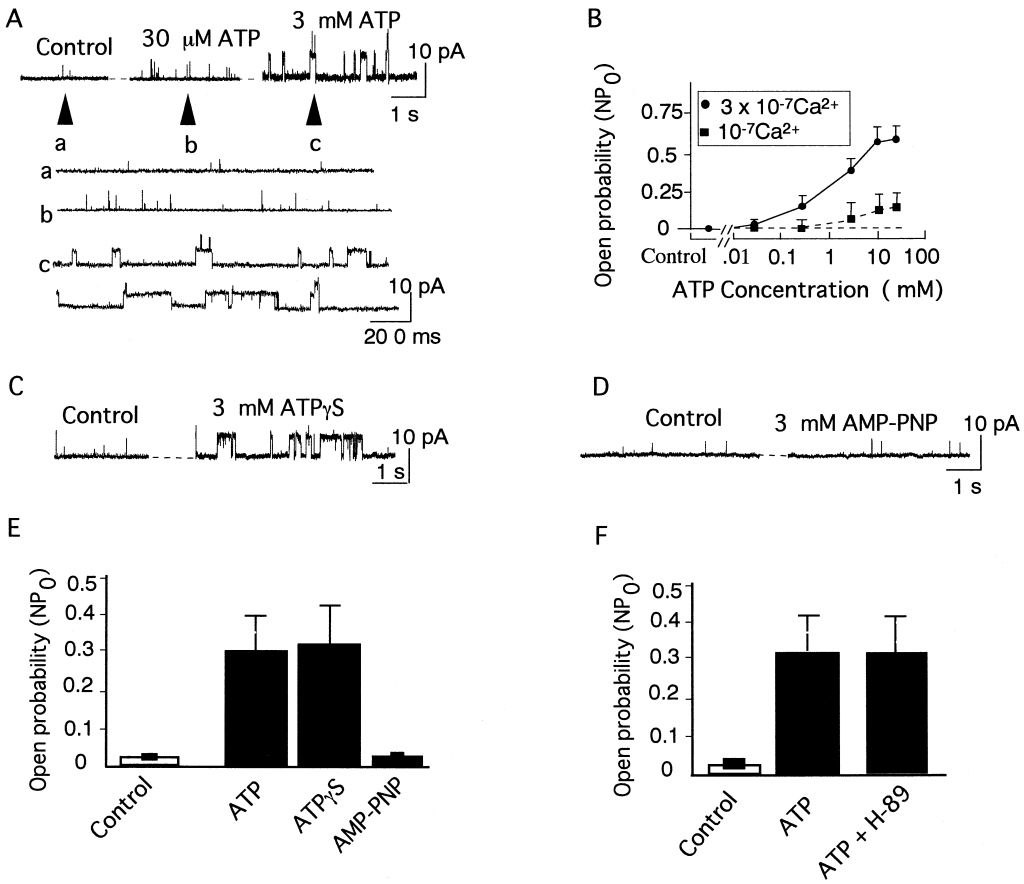

Fig. 2. Modulation of K_{Ca} channel activation by ATP in inside-out patches. The channel currents were recorded under control conditions (trace a) following addition of 30 μM ATP to the bath solution (trace b) and following addition of 3 mM ATP to the bath solution (trace c). K_{Ca} channels were activated significantly following addition of 3 mM ATP to the bath solution. The bath solution contained 100 mM K-aspartate, 40 mM KCl, 10 mM K-MOPS buffer, 1 mM Mg^{2+}, and 3×10^{-7} M Ca^{2+} (in these experiment unless otherwise indicated), pH 7.2. The pipette solution contained 140 mM KCl, 10 mM K-MOPS, and 10^{-4} M Ca^{2+}, pH 7.2. The membrane potential (V_m) was +20 mV in these experiment unless otherwise indicated. (B) Each point represents the mean open probability (NP_o) of K_{Ca} channels which were modulated by ATP in the bath solution containing 10^{-7} M Ca^{2+}(■) or 3×10^{-7} M Ca^{2+}(●). Intracellular application of ATP activated K_{Ca} channels in a dose-dependent manner. (C) Intracellular application of ATPγS to the bath solution. The channel currents were recorded under control conditions and following application of 3 mM ATPγS to the bath solution. (D) Shows that 3 mM AMP-PNP was ineffective in causing K_{Ca} channel activation. The graph shows the effects of ATP and ATP analogues (ATPγS and AMP-PNP) on K_{Ca} channels (E). (F) Shows that K_{Ca} channels were modulated by ATP and subsequent addition of H-89 to the bath solution. The bath solution and pipette solution were the same as in (A).

were suppressed by lowering cytosolic Ca^{2+} with ethyleneglycol-bis-(β-aminoethylether) N,N'-tetraacetic acid (EGTA) (Fig. 1B). The histogram shows the K_{Ca} currents recorded under control conditions and after application of 10^{-6} M Ca^{2+} to the bath solution (Fig. 1C). The I/V curve is given to illustrate the single-channel conductance (215 pS) in Fig. 1D.

3.2. The effects of ATP and ATP analogues on K_{Ca} channels

Intracellular ATP has been shown to modulate the activity of K_{Ca} channels in a number of tissues. We also investigated the intracellular effects of ATP on K_{Ca} channels in chromaffin cells. As depicted in Fig. 2A, application of 30 μM ATP to the cytoplasmic surface of an excised inside-out patch did not lead to significant activation of K_{Ca} channels. However, application of 3 mM ATP to the bath solution led to a large increase in channel activation (NP_o from 0.003 ± 0.001 to 0.300 ± 0.104, $n = 5$). ATP at concentrations of 30 μM to 10 mM applied to the cytosolic side activated K_{Ca} channels in a dose-dependent manner (Fig. 2B and Fig. 4A). When the bath solution contained a lower concentration of Ca^{2+} (10^{-7} M), ATP was needed in higher concentrations (3 to 10 mM) to activate K_{Ca} channels ($n = 6$) (Fig. 2B).

To examine whether ATP hydrolysis was necessary for this effect, analogues of ATP were tested. ATPγS, a thiophosphate analogue of ATP, a complex resistant to dephosphorylation (Kozlowski et al., 1989), at a concentration of 3 mM also activated the K_{Ca} channel significantly (NP_o from 0.004 ± 0.001 to 0.311 ± 0.120, $n = 5$) (Fig. 2C). In contrast to ATP and ATPγS, the non-metabolizable ATP analogue, 5′-adenylylimidodiphosphate (AMP-PNP), failed to mimic ATP in altering K_{Ca} channel activation ($n = 4$) (Fig. 2D), indicating that ATP hydrolysis is required for channel activation. Fig. 2E shows the effect of ATP and the analogues of ATP (ATPγS and AMP-PNP) on K_{Ca} channels.

K_{Ca} channels are modulated by protein phosphorylation. This modulation of K_{Ca} channels is blocked by a specific inhibitor of cyclic AMP-dependent protein kinase (Kume et al., 1989; Esguerra et al., 1994; Lee et al., 1995; Hartley and Kozlowski, 1996). In order to clarify this hypothesis, the effect of staurosporin, a non-specific kinase inhibitor, (Tamaoki et al., 1986), was tested on the ATP-mediated K_{Ca} channel activation (Lee et al., 1995; Bielefeldt and Jackson, 1994). This substance blocks kinase C and is useful to identify co-factor-independent phosphorylation of K_{Ca} channels by members of the kinase C family. H-89, a cAMP-dependent protein kinase inhibitor, (Chijiwa et al., 1990), was tested on the ATP-induced changes in channel activation. H-89 (100 nM) or staurosporine (10 nM) did not induce any change in the open probability of

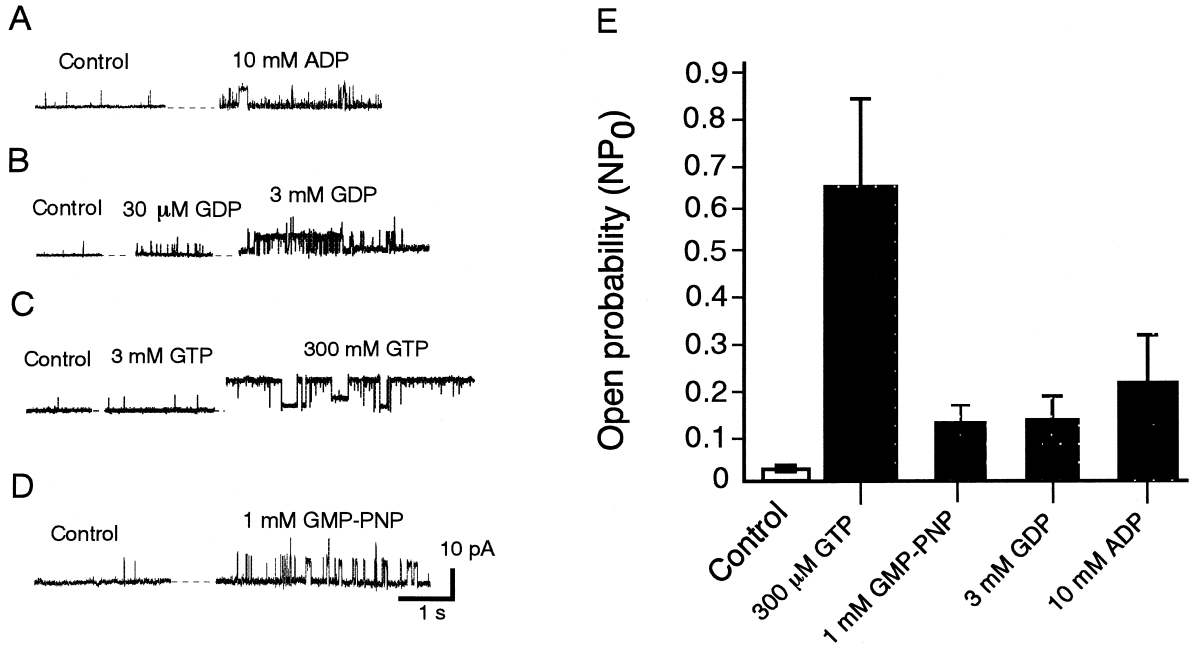

Fig. 3. Stimulatory regulation of K_{Ca} channels by ADP and other nucleotides in inside-out patches. (A) Effects of ADP on K_{Ca} channel activation in inside-out patches. The channel currents were recorded under control conditions and following application of 10 mM ADP to the bath solution. K_{Ca} channels were significantly activated by 10 mM ADP. (B) Effects of GDP on K_{Ca} channel activation in inside-out patches. The channel currents were recorded under control conditions, following addition of 30 μM GDP to the bath solution, and following addition of 3 mM GDP to the bath solution. (C) The channel currents were recorded under control conditions, following addition of 3 μM GTP to the bath solution, and following addition of 300 μM GTP to the bath solution. (D) Effects of GMP-PNP on K_{Ca} channel activation in inside-out patches. K_{Ca} channels were significantly activated by 1 mM GMP-PNP. (E) The graph shows the effects of ADP and other nucleotides on K_{Ca} channels. The bath solution and the pipette solution were the same as in Fig. 2.

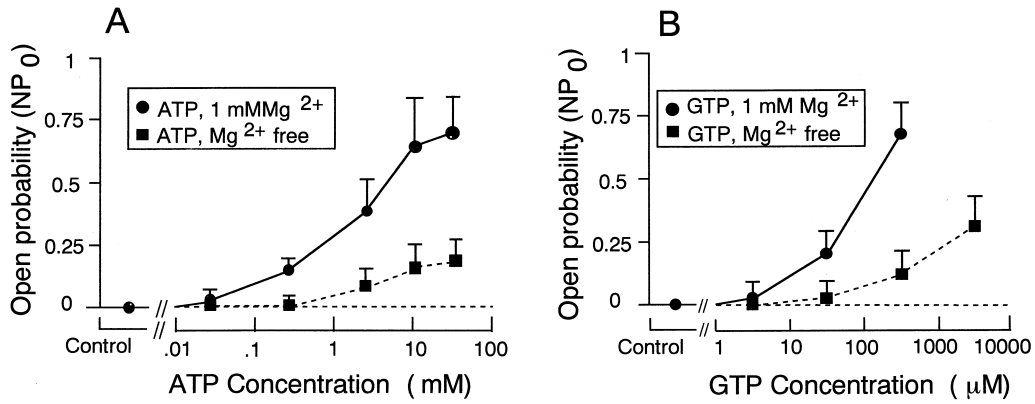

Fig. 4. The concentration-dependent effect of ATP and GTP on K_{Ca} channel activation was studied in inside-out patches. Each curve shows the representative experiment in which K_{Ca} channels were modulated by ATP (A) and GTP (B) in Mg^{2+}-free solution (■) or in the presence of 1 mM Mg^{2+}(●). The bath solution (except Mg^{2+}-free conditions) and pipette solutions were the same as in Fig. 2.

channels activated by ATP ($n = 4$). Fig. 2F shows the effect of H-89 on ATP-activated K_{Ca} channels.

3.3. The effect of ADP and other nucleotides on K_{Ca} channels

Application of ADP and other nucleotides to the cytoplasmic surface of excised inside-out patches led to a large increase in K_{Ca} channel activation (Lee et al., 1995). In the present study, when a high concentration of ADP (10 mM) was added to the cytoplasmic side of the channel, the open probability increased substantially (NP_o from < 0.001 to 0.208 ± 0.104, $n = 4$). The effects of ADP on K_{Ca} channel activation were dependent upon the presence of intracellular Mg^{2+}. To determine whether GTP or GDP could directly activate K_{Ca} channels, we examined the effects of intracellular GTP and GDP on K_{Ca} channel activation. Single-channel activation was observed infrequently under control conditions ($NP_o < 0.001$, $n = 6$). GDP activated K_{Ca} channels in a dose-dependent manner between 30 μM and 3 mM. GDP at a concentration of 3 mM activated K_{Ca} channels significantly (NP_o from 0.003 ± 0.001 to 0.113 ± 0.093, $n = 6$). GTP activated K_{Ca} channels at lower concentrations than did ATP. Application of 300 μM but not 3 μM GTP to the bath solution activated K_{Ca} channels significantly (NP_o from < 0.001 to 0.657 ± 0.169, $n = 6$).

The effects of the non-hydrolyzable derivative of GTP, guanylylimidodiphosphate (GMP-PNP) were also tested. In contrast to AMP-PNP, GMP-PNP (1 mM) also activated K_{Ca} channels significantly (NP_o from < 0.001 to 0.163 ± 0.103, $n = 4$). Fig. 3E summarizes the effects of the nucleotides on K_{Ca} channel activation. The graph shows a representative experiment in which K_{Ca} channels were modulated by GTP, GMP-PNP, GDP and ADP.

3.4. The effects of Mg^{2+} on nucleotide-induced activation of K_{Ca} channels

In the presence of millimolar Mg^{2+}, ATP is largely complexed with Mg^{2+}. To test whether free ATP or its Mg^{2+} complex is the active agonist, we also studied the concentration dependence of the response to ATP and other nucleotides in the absence of intracellular Mg^{2+}. The ATP response approached saturation at 10 mM and the concentration of ATP that elicited 50% of the maximal response (EC_{50}) was 1.5 mM (Fig. 2BFig. 4). In the absence of Mg^{2+}, ATP and the other nucleotides had to be used in higher concentrations to activate K_{Ca} channels ($n = 6$). The EC_{50} of the response to uncomplexed ATP was 5 mM, considerably higher than that for complexed ATP, Mg-ATP (1.5 mM). The shift in the curve for uncomplexed ATP suggests that free ATP, and not complexed ATP could modulate K_{Ca} channels but to a lesser extent (Fig. 4).

To determine whether Mg^{2+} alone could activate K_{Ca} channels, we added 1 mM Mg^{2+} to the bath solution (cytosolic side) in inside-out patches. Mg^{2+} did not induce any change in the open probability when applied alone ($n = 6$, data not shown).

4. Discussion

Secretory vesicles of chromaffin cells store catecholamines and ATP in high concentrations (Winkler, 1976; Westhead and Winkler, 1982). ATP is co-secreted and co-released with catecholamines following physiologic stimulation (Rojas et al., 1985) and can induce both inhibition and enhancement of the Ca^{2+} current (Diverse et al., 1991). The ATP-induced rise in $[Ca^{2+}]_i$ is due to the release of Ca2 + from internal stores and entry across the plasma membrane; the latter source of Ca2 + initiates secretion (Kim and Westhead, 1989). These Ca2 + currents are inactivated by increased concentrations of ATP and intracellular Ca2 + (Otsuguro et al., 1996).

Ion channels which are activated by intracellular Ca2 + ions have been found in various cell types (Marty, 1989). However, previous reports indicate that Cl − channel activation dose not appear to be related to $[Ca2 +]_i$ increase

(Hazama and Okada, 1988; Doroshenko et al., 1991; Doroshenko and Neher, 1992) and that Mg-ATP and GTP by themselves decrease channel activation. In this study, we found that Ca2 + at the intracellular membrane surface activates KCa channels and that this channel could be blocked by intracellular application of tetraethylammonium or EGTA. Tetraethylammonium is an effective inhibitor of the K_{Ca} channel when applied on either side of the membrane. These results demonstrate that the channels examined were indeed K_{Ca} channels. But the K_{Ca} channel is insensitive to external or internal charybdotoxin. The previous reports has shown two types of K_{Ca} channels from rat brain modulated by different sets of enzymes (Bielefeldt et al., 1992; Chung et al., 1991), i.e., a channel referred to as type 1, which was charybdotoxin sensitive and the other referred to as type 2, which was charybdotoxin insensitive (Reinhary et al., 1991; Wang et al., 1992). The channel in the present study was not inhibited by charybdotoxin, indicating that ATP might directly activate the charybdotoxin-insensitive K_{Ca} (type II) channel in chromaffin cells.

K_{Ca} channels are recognized as playing a vital role in controlling the membrane potential of a variety of cell types (Brayden and Nelson, 1992). In chromaffin cells, K_{Ca} channels may be the crucial regulatory link between $[Ca^{2+}]_i$ and catecholamine secretion (Petersen and Maruyama, 1984; Uceda et al., 1992). K_{Ca} channel activation is of particular interest because it provides explanations for several well-known phenomena in which the export of K^+ from cells appears to be linked to changes in cell metabolism and membrane potential (Petersen and Maruyama, 1984). Inhibition of K_{Ca} channels results in gating of voltage-dependent Ca^{2+} channels and thereby augments catecholamine secretion (Sorimachi et al., 1990; Gonzalez-Garcia et al., 1993). Activation of K_{Ca} channels leads to membrane hyperpolarization, which impedes further Ca^{2+} influx (Meech and Standen, 1975; Gorman and Thomas, 1980; Petersen and Maruyama, 1984). The cellular regulation of K_{Ca} channel activation is still not well understood. However, only a few reports exist which suggest that these channels possess the capacity for autophosphorylation as a consequence of endogenous kinase activity or through a kinase firmly associated with this channel at the membrane level (Chung et al., 1991; Lee et al., 1995; Bielefeldt and Jackson, 1994; Esguerra et al., 1994). On the basis of our findings when using staurosporine and H-89, intracellular ATP-induced cell membrane hyperpolarization via activation of K_{Ca} channels is not modulated by the closely associated kinase.

Our results suggest that the K_{Ca} channels found in chromaffin cells are activated directly by physiological levels of ATP. This modulation takes place without the addition of protein kinase and is mimicked by an ATP analogue (ATPγS), but not by a non-hydrolyzable ATP analogue (AMP-PNP). These findings are consistent with the hypothesis that intracellular ATP can modulate K_{Ca} channels directly, and that phosphorylation can occur in excised patches (Bielefeldt and Jackson, 1994). But there are several important differences between their studies and ours. Firstly, ATP-mediated K_{Ca} channel activation is not due to the activity of a closely-associated, membrane-bound kinase. Secondly, although many biological processes which utilize ATP as a high-energy phosphate donor require the divalent cation Mg^{2+} as a co-factor, it appears that free ATP and not complexed ATP, is effective in altering the activity of K_{Ca} channels (although to a smaller extent). Moreover, ATPγS was used as a non-hydrolyzable ATP analogue; however, this nucleotide possesses a terminal thiophosphate bond and has been reported to substitute for ATP in many processes which require phosphorylation. ATPγS has been reported to have variable effects upon ATP-activated K_{Ca} channels. In cortical synaptosomes ATPγS produced a larger increase in channel activation than did ATP (Chung et al., 1991), whereas in rat cortical neurons ATPγS modulated K_{Ca} channels to a variable extent (it was much less potent than ATP or produced a large increase in channel activation) (Lee et al., 1995). In the present study, the activation of K_{Ca} channels by ATPγS was very similar to that elicited by ATP. The result obtained with the ATP analogue suggests a role for protein phosphorylation in the upregulation of K_{Ca} channel activity. We do not know if the effect is mediated by a direct binding effect after phosphorylation or/and binding of the nucleotides to the channel directly.

The genes encoding K_{Ca} channels were found in the muscles of *Drosophila* slowpoke (Atkinson et al., 1991) and a putative nucleotide binding site has also been identified. Whether this sequence in the K_{Ca} channel serves as a site for G protein regulation or as a site of direct nucleotide (GTP or ATP) binding, has not been addressed. Recently, Kenneth et al. (1996) found that K_{Ca} channels could be directly modulated by G protein in bovine adrenal chromaffin cells. In this study, activation of K_{Ca} channel by cytoplasmic GMP-PNP might be mediated by G proteins in chromaffin cells.

ATP serves as a negative feedback inhibitor of voltage-gated Ca^{2+} channel currents in cultured bovine adrenal chromaffin cells and as such is an important regulator of catecholamine secretion (Currie and Fox, 1996). The results of the present study suggest that cytosolic ATP and other nucleotides induce cell membrane hyperpolarization through activation of K_{Ca} channels by dual pathways: one with, and the other without, protein phosphorylation.

References

Albarwani, S., Robertson, B.E., Nye, P.C., Kozlowski, R.Z., 1994. Biophysical properties of Ca^{2+}- and Mg-ATP-activated K^+ channels in pulmonary arterial smooth muscle cells isolated from the rat. Pflüg. Arch. 428, 446–454.

Artalejo, A.R., Garcia, A.G., Neher, E., 1993. Small-conductance Ca^{2+}-activated K^+ channels in bovine chromaffin cells. Pflüg. Arch. 423, 97–103.

Asano, T., Otsuguro, K., Ohta, T., Sugawara, T., Nakazato, Y., 1995. Characterization of ATP-induced catecholamine secretion from adrenal chromaffin cells of the guinea-pig. Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 112, 101–108.

Atkinson, N.S., Robertson, G.A., Ganetzky, B., 1991. A component of calcium-activated potassium channels encoded by the *Drosophila* slo locus. Science 253, 551–555.

Bielefeldt, K., Jackson, M.B., 1994. Phosphorylation and dephosphorylation modulate a Ca^{2+}-activated K^+ channel in rat peptidergic nerve terminals. J. Physiol. 475, 241–254.

Bielefeldt, K., Rotter, J.L., Jackson, M.B., 1992. Three potassium channels in rat posterior pituitary nerve endings. J. Physiol. 458, 41–67.

Brayden, J.E., Nelson, M.T., 1992. Regulation of arterial tone by activation of calcium-dependent potassium channels. Science 256, 532–535.

Chern, Y.J., Herrera, M., Kao, L.S., Westhead, E.W., 1987. Inhibition of catecholamine secretion from bovine chromaffin cells by adenine nucleotides and adenosine. J. Neurochem. 48, 1573–1576.

Chijiwa, T., Mishima, A., Hagiwar, M., Sano, M., Hayashi, K., Inoue, T., Naito, K., Toshioka, T., Hidaka, H., 1990. Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, *N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma cells. J. Biol. Chem. 265, 5267–5272.

Chung, S.K., Reinhart, P.H., Martin, B.L., Brautigan, D., Levitan, I.B., 1991. Protein kinase activity closely associated with a reconstituted calcium-activated potassium channel. Science 253, 560–562.

Currie, K.P., Fox, A.P., 1996. ATP serves as a negative feedback inhibitor of voltage-gated Ca^{2+} channel currents in cultured bovine adrenal chromaffin cells. Neuron 16, 1027–1036.

Diverse, P.M., Dunlap, K., Westhead, E.W., 1991. Multiple actions of extracellular ATP on calcium currents in cultured bovine chromaffin cells. Proc. Natl. Acad. Sci. USA 88, 1261–1265.

Doroshenko, P., Neher, E., 1992. Volume-sensitive chloride conductance in bovine chromaffin cell membrane. J. Physiol. 449, 197–218.

Doroshenko, P., Penner, R., Neher, E., 1991. Novel chloride conductance in the membrane of bovine chromaffin cells activated by intracellular GTP gamma S. J. Physiol. 436, 7111–7124.

Esguerra, M., Wang, J., Foster, C.D., Adelman, J.P., North, R.A., Levitan, I.B., 1994. Cloned Ca^{2+}-dependent K^+ channel modulated by a functionally associated protein kinase. Nature 369, 563–565.

Gandia, L., Garcia, A.G., Morad, M., 1993. ATP modulation of calcium channels in chromaffin cells. J. Physiol. 470, 55–72.

Gonzalez-Garcia, C., Cena, V., Keiser, H.R., Rojas, E., 1993. Catecholamine secretion induced by tetraethylammonium from cultured bovine adrenal chromaffin cells. Biochim. Biophys. Acta 1177, 99–105.

Gorman, A.L., Thomas, M.V., 1980. Potassium conductance and internal calcium accumulation in a molluscan neuron. J. Physiol. 308, 287–313.

Greenberg, A., Zinder, O., 1982. Alpha- and beta-receptor control of catecholamine secretion from isolated adrenal medulla cells. Cell Tissue Res. 226, 655–665.

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.G., 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflüg. Arch. 391, 85–100.

Hartley, S.A., Kozlowski, R.Z., 1996. ATP increases Ca^{2+}-activated K^+ channel activity in isolated rat arterial smooth muscle cells. Biochim. Biophys. Acta 1283, 192–198.

Hazama, A., Okada, Y., 1988. Ca^{2+} sensitivity of volume-regulatory K^+ and Cl^- channels in cultured human epithelial cells. J. Physiol. 402, 687–702.

Kenneth, B., Walsh, S.P., Wilson, K.J., Long, S.C., 1996. Stimulatory regulation of the large-conductance, calcium-activated potassium channel by G protein in bovine adrenal chromaffin cells. Mol. Pharmacol. 49, 379–386.

Kim, K.T., Westhead, E.W., 1989. Cellular responses to Ca^{2+} from extracellular and intracellular sources are different as shown by simultaneous measurements of cytosolic Ca^{2+} and secretion from bovine chromaffin cells. Proc. Natl. Acad. Sci. USA 86, 9881–9885.

Kozlowski, R.Z., Hales, C.N., Ashford, M.L.J., 1989. Dual effects of diazoxide on ATP-K^+ currents recorded from an insulin-secreting cell line. Br. J. Pharmacol. 97, 1039–1050.

Kume, H., Takai, A., Tokuno, H., Tomita, T., 1989. Regulation of Ca^{2+}-dependent K^+-channel activity in tracheal myocytes by phosphorylation. Nature 341, 152–154.

Lee, K., Rowe, I.C., Ashford, M.L., 1995. Characterization of an ATP-modulated large conductance Ca^{2+}-activated K^+ channel present in rat cortical neurones. J. Physiol. 488, 319–337.

Marty, A., 1981. Ca^{2+}-dependent K^+ channels with large unitary conductance in chromaffin cell membranes. Nature 291, 497–500.

Marty, A., 1989. The physiological role of calcium-dependent channels. Trends Neurosci. 12, 402–424.

Meech, R.W., Standen, N.B., 1975. Potassium activation in Helix aspersa neurones under voltage clamp: a component mediated by calcium influx. J. Physiol. 249, 211–259.

Marty, A., Neher, E., 1985. Potassium channels in cultured bovine adrenal chromaffin cells. J. Physiol. 367, 117–141.

Otsuguro, K.I., Ohta, T., Ito, S., Nakazato, Y., 1996. Modulation of calcium current by ATP in guinea-pig adrenal chromaffin cells. Pflug. Arch. 431, 402–407.

Petersen, O.H., Maruyama, Y., 1984. Calcium-activated potassium channels and their role in secretion. Nature 307, 693–697.

Reichsman, F., Santos, S., Westhead, E.W., 1995. Two distinct ATP receptors activate calcium entry and internal calcium release in bovine chromaffin cells. J. Neurochem. 65, 2080–2086.

Reinhary, P.H., Chung, S., Martin, B.L., Brautigan, D., Levitan, I.B., 1991. Modulation of calcium-activated potassium channels from rat brain by protein kinase A and protein phosphatase 2A. J. Neurosci. 11, 1627–1635.

Rojas, E., Pollard, H.B., Heldman, E., 1985. Real-time measurements of acetylcholine-induced release of ATP from bovine medullary chromaffin cells. FEBS Lett. 185, 323–327.

Sorimachi, M., Yamagami, K., Nishimura, S., 1990. Tetraethylammonium stimulates adrenomedullary secretion by causing fluctuations in a cytosolic free Ca concentration. Brain Res. 507, 347–350.

Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M., Tomita, F., 1986. Staurosporine, a potent inhibitor of phospholipid/Ca^{2+} dependent protein kinase. Biophys. Biochim. Res. Commun. 135, 397–402.

Uceda, G., Artalejo, A.R., Lopez, M.G., Abad, F., Neher, E., Garcia, A.G., 1992. Ca^{2+}-activated K^+ channels modulate muscarinic secretion in cat chromaffin cells. J. Physiol. 454, 213–230.

Wang, X., Thorn, P.J., Lemos, J.R., 1992. A novel large-conductance calcium-activated potassium channel and current in never terminals of the rat neurohypophysis. J. Physiol. 457, 47–74.

Westhead, E.W., Winkler, H., 1982. The topography of gangliosides in the membrane of the chromaffin granule of bovine adrenal medulla. Neuroscience 7, 1611–1614.

Winkler, H., 1976. The composition of adrenal chromaffin granules: an assessment of controversial results. Neuroscience 1, 65–80.

Yellen, G., 1984. Ionic permeation and blockade in Ca^{2+}-activated K^+ channels of bovine chromaffin cells. J. Gen. physiol. 84, 157–186.