*Journal of Bioenergetics and Biomembranes, Vol. 23, No. 4, 1991*

# MINI-REVIEW

# Calcium-Activated $K^+$ Channels: Metabolic Regulation

L. Toro[1] and E. Stefani[1]

*Received June 4, 1990*


## Abstract

Calcium-activated potassium ($K_{Ca}$) channels are highly modulated by a large spectrum of metabolites. Neurotransmitters, hormones, lipids, and nucleotides are capable of activating and/or inhibiting $K_{Ca}$ channels. Studies from the last few years have shown that metabolites modulate the activity of $K_{Ca}$ channels via: (1) a change in the affinity of the channel for $Ca^{2+}$ ($K_{1/2}$ is modified), (2) a parallel shift in the voltage axis of the acitvation curves, or (3) a change in the slope (effective valence) of the voltage dependence curve. The shift of the voltage dependence curve can be a direct consequence of the change in the affinity for $Ca^{2+}$. Recently, the mechanistic steps involved in the modulation of $K_{Ca}$ channels are being uncovered. Some interactions may be direct on $K_{Ca}$ channels and others may be mediated via G-proteins, second messengers, or phosphorylation. The information given in this review highlights the possibility that $K_{Ca}$ channels can be activated or inhibited by metabolites without a change in the intracellular $Ca^{2+}$ concentration.


**Key Words**: Calcium-activated potassium channels; modulation; metabolic regulation; neurotransmitters; hormones; lipids; nucleotides.

## Introduction

Calcium-activated potassium channels ($K_{Ca}$) comprise a large family of K channels of various conductances, voltage dependences, calcium sensitivities, and pharmacological profiles (for a recent review, see Latorre *et al.*, 1989). Besides their regulation by voltage and calcium, there is now increasing evidence that they are also subject to multiple metabolic regulation. Basic

[1]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas 77030.



0145-479X/91/0800-0561$06.50/0 

and encouraging contributions were made in studies where calcium-activated K conductances were proposed to be down-regulated by noradrenaline and histamine, as judged by spike activity and afterhyperpolarization of neurons (Madison and Nicoll, 1982; Haas and Konnerth, 1983). Since then, a growing list of metabolites (neurotransmitters, hormones, lipids, and nucleotides) that modify the activity of $K_{Ca}$ channels or currents has been uncovered (Table I). An insight into the mechanism(s) by which metabolic regulation takes place at the single-channel level has been obtained and is fundamental in understanding the physiological role of $K_{Ca}$ channels. The patch clamp technqiue has been invaluable for this purpose, but has the disadvantage that multiple cellular constituents are present and a clear-cut dissection of the mechanism(s) is difficult. We have used another approach which is the reconstitution of channels into bilayers, which allows stricter control of the environment of the channel. However, more accurate answers may possibly be obtained studying purified channels and purified "modulators".

As we shall discuss in this review, it is plausible that several metabolic pathways may affect one channel, making the responses more varied and rendering $K_{Ca}$ channels versatile. We expect that in a few years metabolic charts (as those for metaboilc pathways) will be necessary to summarize the known pathways by which channels are regulated.

## Neurotransmitters and Hormones

### *Antidiuretic Hormone (ADH)*

$K_{Ca}$ channels (107 pS) from cultured kidney cells are activated by ADH[2], showing an increase in their fractional open time (Guggino *et al.*, 1985). The mechanism(s) or metabolic route(s) by which this hormone activates kidney $K_{Ca}$ channels is beginning to be understood. Cell-attached patches showed that $K_{Ca}$ channels could be activated by applying ADH in the bathing solution. Under these conditions (because of the high resistance of the seal) any drug that is applied in the bathing media is not able to reach the channel(s) in the patch. Therefore, it may be hypothesized that ADH was not activating $K_{Ca}$ channels directly but via second messenger(s). In fact, the authors found that forskolin (10 $\mu$M), an activator of adenylate cyclase,

[2]Abbrevations: ADH, antidiuretic hormone; PLA, c-AMP dependent protein kinase; ANF, atrial natriuretic factor; $P_o$, open probability; $IP_3$, inositol-1,4,5-trisphosphate; $IP_4$, 1,3,4,5-tetrakisphosphate; $[Ca^{2+}]_i$, internal calcium concentration; STOCs, spontaneous transient outward currents; G-proteins, GTP-dependent proteins; $G_s$, G-protein coupled to adenylate cyclase; $G_{plc}$, G-protein coupled to phospholipase C; $G_{pla}$, G-protein coupled to phospholipase $A_2$.

could increase channel activity. Based on biochemical studies which indicate that ADH increases cAMP-dependent protein kinase (PKA) (Edwards *et al.*, 1980), it was concluded that a possible mechanism could be phosphorylation of the channel itself or of a related "modulator" of the channel. It is interesting to note that a larger degree of activation and a slower time response (50 s vs. 2 min) was observed when forskolin was the activator and not ADH. Several questions and possible answers arise: (1) Does the response due to ADH involve more steps [perhaps a G-protein (GTP-dependent protein) activation] than merely activation of adenylate cyclase, explaining its lag time of action? It is known that activation of a class of G-proteins ($G_s$-protein), catalyzed by the agonist–receptor interaction, triggers adenylate cyclase (Gilman, 1987; Birnbaumer *et al.*, 1987). Thus, it is possible that ADH activation of $K_{Ca}$ channels follows this route. (2) If G-proteins are involved, is the activation of adenylate cyclase the only action of G-proteins and/or they themselves are activating $K_{Ca}$ channels acting directly on the channels or another "modulator"? It is known that G-proteins can directly modulate ionic channels (see Brown in this series; Brown and Birnbaumer, 1990), and evidence exists that $K_{Ca}$ currents (Cole and Sanders, 1989) and channels may also be their targets (Toro *et al.* 1990a). (3) Is the action of G-proteins or of the "modulator" antagonic to the action of cAMP, explaining a smaller activation by ADH? (4) Is cAMP acting directly on the channel, or does it trigger PKA leading to phosphorylation of the channel or of the "modulator"? This possibility may also be truth since activation by phosphorylation of $K_{Ca}$ channels by PKA has been demonstrated in *Helix aspersa* (Ewald *et al.*, 1985) and in aortic (Sadoshima *et al.*, 1988) and tracheal myocytes (Kume *et al.*, 1989).

### *Gonadotropin-Releasing Hormone (GnRH)*

$K_{Ca}$ channels (> 50 pS) from gonadotrophs of ovine pituitary (Mason and Waring, 1986) and pars tuberalis (Sikdar *et al.*, 1989) are activated by GnRH (100 ng/ml and 10 $\mu$g/ml). GnRH applied extracellularly to cell-attached patches increased the probability of the channel being in the open state. As discussed before, this result indicates that GnRH was not gating the channel directly, but an intermediary(ies) was involved. Based on previous studies (Mason and Waring, 1986; Shangold *et al.*, 1988), the increment in activity of $K_{Ca}$ channels was attributed to a rise in the internal free calcium concentration ($[Ca^{2+}]_i$), after GnRH stimulation. Sikdar *et al.* (1989) also investigated the action of cAMP and phosphorylation on $K_{Ca}$ channels since it was known that cAMP increased GnRH-induced secretion of LH (luteinizing hormone (Turgeon and Waring, 1986). Surprisingly, addition of dibutyryl cAMP (a membrane-permeant analog of cAMP) or forskolin to cell-attached

**Table I.** Modulation of $K_{Ca}$ Channels in Different Cell Types

| Metabolite | Cell type | Conductance (pS) | Activation/inhibition (+/−) | Sensor affected ($V/Ca^{2+}$)[a] | Reference |
|---|---|---|---|---|---|
| | | Neurotransmitters and hormones | | | |
| Antidiuretic hormone | Kidney | 107 | + | -- | Guggino *et al.*, 1985 |
| Gonadoropin-releasing hormone | Gonadotrophs | > 50 | + | -- | Mason and Waring, 1986; Sikdar *et al.*, 1989 |
| Substance P | Smooth muscle | ≈ 200 | +/− | -- | Mayer *et al.*, 1989; 1990 |
| Bradykinin | Neuroblastoma--glioma hybrid cells | -- | + | -- | Higashida and Brown, 1986 |
| Histamine | Neurons | -- | + | -- | Haas and Konnerth, 1983 |
| | HeLa cells | 50 | + | -- | Sauvé *et al.*, 1987 |
| | Smooth muscle | -- | − | -- | Neliat *et al.*, 1989 |
| Acetylcholine | Lacrimal cells | -- | + | -- | Morris *et al.*, 1987 |
| | Smooth muscle | ≈ 200 | − | V | Cole *et al.*, 1989 |
| Adrenergic agents | Neurons | -- | − | -- | Madison and Nicoll, 1982; Haas and Konerth, 1983 |
| | Smooth muscle | 120–260 | + | $V/Ca^{2+}$ | Sadoshima *et al.*, 1988; Kume *et al.*, 1989; Ramos-Franco *et al.*, 1989, 1990; Toro *et al.*, (1990a) |
| Angiotensin II | Smooth muscle | ≈ 250 | − | -- | Toro, *et al.*, 1990b |
| Adenosine and atrial natriuretic factor | Smooth muscle | 266 | + | $V/Ca^{2+}$ | Williams *et al.*, 1988 |

| | | | | | |
|---|---|---|---|---|---|
| | | Lipid sand metabolites | | | |
| Arachidonic acid and fatty acids | Smooth muscle | ≈200 | + | -- | Ordway *et al.*, 1989; Katz *et al.*, 1990 |
| Cholesterol | Smooth muscle | 300 | + | -- | Bolotina *et al.*, 1989 |
| | | Nucleotides | | | |
| ADP | Oocytes | 40–85 | + | V | Yoshida *et al.*, 1990 |
| GMP | Smooth muscle | 266 | + | $V/Ca^{2+}$ | Williams *et al.*, 1988 |
| cGMP, GDP, GTP | Smooth muscle | 266 | + | -- | Williams *et al.*, 1988 |

[a] V, voltage; dashed lines, not available.

patches induced $K_{Ca}$ channel inhibition. This inhibition could be due to: (1) cAMP acting on the channel, or (2) phosphorylation of the channel itself or of a closely associated "modulator." The authors found that the action of cAMP (1 mM) in inside-out patches required 1 mM ATP and $Mg^{2+}$ to take place. This suggested to them the presence of an endogenous PKA in the isolated patch. This assumption was explored by adding a protein kinase inhibitor (P5636) to the internal side of the inside-out patch in the presence of 10 $\mu$M cAMP and 1 mM Mg-ATP. This maneuver caused an increase in $P_o$, strongly suggesting both the presence of an endogenous PKA in the isolated patch and a phosphorylation mechanism involved in the inhibition of $K_{Ca}$ channels. Accordingly, $K_{Ca}$ channels were also inhibited by the catalytic subunit of PKA applied to the intracellular side of outside-patches.

This is an example of $K_{Ca}$ channels which are modulated by more than one intracellular metabolic pathway before a final physiological response is attained, in this case the secretion of LH. Multiple questions on the metabolic cascade by which GnRH activates $K_{Ca}$ channels remain open, for example: (1) Is the rise of internal $Ca^{2+}$ mediated by GnRH the only means of positive modulation from gonadotrophs $K_{Ca}$ channels? The increase in $[Ca^{2+}]_i$ is due to both extrusion of $Ca^{2+}$ from internal sources and influx via plasmalemmal $Ca^{2+}$ channels (Mason and Waring, 1986; Shangold *et al.*, 1988). It is known in other systems that extrusion of $Ca^{2+}$ from internal sources by inositol-1,4,5-trisphosphate ($IP_3$) follows the sequence: (i) agonist–receptor interaction → catalytic activation of $G_p$-proteins ($G_p$) → activation of phospholipase C → production of $IP_3$ + diacylglycerol; (ii) $IP_3$ activates $Ca^{2+}$ release channels from internal sources, and diacylglycerol activates the phospholipid-$Ca^{2+}$ dependent protein kinase (protein kinase C) (Birnbaumer *et al.*, 1987, 1989). From this sequence, other logical candidates that might activate $K_{Ca}$ channels from gonadotrophs and should be explored are $IP_3$ and G-proteins.

### *Substance P*

$K_{Ca}$ channels ($\approx$ 200 pS) from colonic smooth muscle (Carl and Sanders, 1989; Mayer *et al.*, 1989) are differentially modulated by substance P depending on the peptide concentration (Mayer *et al.*, 1989). In cell-attached patches, low concentrations of substance P ($1 \times 10^{-12}$ M) promoted the simultaneous activation of multiple $K_{Ca}$ channels, while higher concentrations ($1 \times 10^{-7}$ M) promoted a transient activation followed by inhibition (desensitization?). Both effects were dependent on external $Ca^{2+}$. However, 1 $\mu$M nifedipine (an L-type $Ca^{2+}$ channel blocker) (Bean, 1989) only inhibited the low-concentration response. Since the cell-attached mode was used in these experiments, it was very likely that substance P was not acting directly on $K_{Ca}$

channels, but indirectly through pathways that raised $[Ca^{2+}]_i$. Recently, Mayer *et al.* (1990) have shown that the low-concentration ($10^{-12}$–$10^{-10}$ M) response (opening of "clusters" of channels) is primarily due to the increase in $Ca^{2+}$ currents (through L-type $Ca^{2+}$ channels) after the interaction between substance P and its receptor (neurokinin-1 type). It is very likely that other mechanism(s) leading to a rise in $[Ca^{2+}]_i$ are also present, since at positive potentials (where $Ca^{2+}$ currents diminish) substance P kept activating $K^+$ currents. Some questions may be: (1) Is the interaction between substance P and the receptor catalyzing the activation of G-proteins followed by activation of a membrane phospholipase? As discussed before, candidates of this metabolic route that may modulate an intracellular site of $K_{Ca}$ channels are $IP_3$, G-proteins, and/or a phosphorylation reaction.

### *Bradykinin*

NG108-15 neuroblastoma-glioma hybrid cells (Higashida and Brown, 1986) elicit an apamin-sensitive $K_{Ca}$ current upon stimulation with bradykinin ($\approx 10\,\mu M$). This $K_{Ca}$ current is also elicited by intracellular iontophoretic injection of $Ca^{2+}$ or $IP_3$. These results suggested to the authors that $IP_3$ was activating a $K_{Ca}$ current by increasing $[Ca^{2+}]_i$. In this scheme, the binding of bradykinin should activate a phospholipase C and produce $IP_3$ and diacylglycerol. In parallel studies, the authors attributed to diacylglycerol the role of inhibiting an M-current. Since it is now known that stimulation of phospholipase C by bradykinin may be via a $G_{plc}$-protein (Birnbaumer *et al.*, 1989), it would be interesting to study the effect of $G_{plc}$-protein on this type of $K_{Ca}$ channels. Questions arise with regard to the regulation at the single-channel level, for example, whether these channels are modulated directly by $IP_3$ or by phosphorylation through the phospholipid (diacylglycerol)-dependent protein kinase (PKC).

### *Histamine*

In early studies in neurons, it was found that a $K_{Ca}$ conductance (responsible for the late hyperpolarization after a burst of spikes) was inhibited by histamine via $H_2$ receptors (Haas and Konnerth, 1983).

Later, $H_1$ receptors have been identified as extracellular targets followed by activation of $K_{Ca}$ channels (Sauvé *et al.*, 1987) or currents (Neliat *et al.*, 1989). Sauvé *et al.*, (1987) found in cell-attached patches that $K_{Ca}$ channels (50 pS at negative potentials; voltage insensitive) from HeLa cells (an epithelial cell line) may be modulated by histamine ($\geqslant 10\,\mu M$) in a dose-dependent manner. Channel activation by histamine was characterized by cycles of burst activity followed by periods of "silence." The authors attributed the silent periods to a decrease in $[Ca^{2+}]_i$. This conclusion was reached after making

an inside-out patch, during a period of silence, and observing full activity of the channel (0.4 $\mu$M external $Ca^{2+}$). The initial burst activity due to histamine activation was not dependent on external $Ca^{2+}$ and could not be mimicked by addition of dibutyryl cAMP to the external solution. Thus, it was concluded that the histamine-evoked periodic activity was due to changes in $[Ca^{2+}]_i$ related to intracellular sources, and that a cAMP-dependent process was not directly responsible for the stimulation of HeLa $K_{Ca}$ channels. As the authors proposed, histamine modulation was unlikely to have a direct effect on the channel, and a metabolic pathway involving $IP_3$ releasing $Ca^{2+}$ from internal sources was a suitable candidate.

Histamine is also capable of inhibiting spontaneous transient outward currents (STOCs) from ear artery smooth muscle (Neliat *et al.*, 1989). STOCs have been attributed to a simultaneous activation of multiple "maxi" $K_{Ca}$ channels from smooth muscle in response to a periodical release of $Ca^{2+}$ from intracellular stores (Benham and Bolton, 1986). Inhibition of STOCs by histamine (0.3–30 $\mu$M) takes place after its interaction with $H_1$ receptors, since the effect was antagonized by the $H_1$ antagonist pyrilamine (1 $\mu$M). Since $H_1$ receptors are known to be coupled to a $G_{plc}$-protein in smooth muscle (Birnbaumer *et al.*, 1989), it would be interesting to test if the known metabolites or possible lateral reactions of this cascade affect vascular smooth muscle $K_{Ca}$ channels at the single-channel level. In the following sequence we have highlighted possible modulators besides $Ca^{2+}$: **Histamine** → $H_1$ receptors → **$G_{plc}$** → **$IP_3$ + diacylglycerol**; {$IP_3$ → release $Ca^{2+}$}; {diacylglycerol → activation of PKC → **phosphorylation**}.

### *Acetylcholine (ACh)*

$K_{Ca}$ channels from colonic smooth muscle are an example of channel multi-regulation by different metabolites in the same cell. These $K_{Ca}$ channels may not only be activated or down-regulated by substance P (see previous paragraph; Mayer *et al.*, 1989) but are also inhibited by ACh (Cole *et al.*, 1989). Muscarinic inhibition of $K_{Ca}$ channels was observed in cell-attached patches when ACh (10 $\mu$M) was present in the bath and pipette solutions. This inhibition was reflected as a shift toward more positive potentials of the voltage-dependent activation curve (Cole *et al.*, 1989). Interestingly, this inhibition was not present in off-cell (inside-out) patches with ACh in the pipette (Cole *et al.*, 1989) or in cell-attached patches if ACh was only applied to the bath but not to the pipette (see Discussion in Cole and Sanders, 1989). These experiments point out that in off-cell patches some essential internal component(s) were missing under these conditions, preventing muscarinic inhibition to take place. In addition, cell-attached experiments suggested that inhibition could not take place if the muscarinic receptor was not "close" to

the channel. Additional observations in whole cell $K_{Ca}$ currents indicate that muscarinic inhibition of these currents is mediated by a G-protein dependent process (Cole and Sanders, 1989). The ACh response of colonic $K_{Ca}$ currents required GTP (100 $\mu$M) or nonhydrolyzable GTP analogs (GTP$\gamma$S and 5′-guanylylimidodiphosphate; 100 $\mu$M) in the pipette, and was suppressed by dialysis of the intracellular milieu with pertussis toxin (1 ng/ml). Former single channel experiments may be explained on the basis of a G-protein mediated muscarinic inhibition. The lack of muscarinic effect in inside-out patches with ACh in the pipette may be due to the absence of GTP in the external solution (Cole *et al.*, 1989) preventing activation of the G-protein. The need of ACh in the patch pipette in cell-attached patches suggests that: (1) after the ACh–muscarinic receptor interaction, a closely related G-protein is activated, gating the channel in a membrane-delimited fashion that may be "direct and obligatory" as for the muscarinic atrial $K^+$ channels (Brown and Birnbaumer, 1990); (2) that a diffusible second messenger produced after G-protein activation was not involved in the inhibition of $K_{Ca}$ channels.

ACh is also known to activate a $K_{Ca}$ current in lacrimal acinar cells (Morris *et al.*, 1987). The authors found that the ACh response is emulated only if $IP_3$ and $IP_4$ (1,3,4,5-tetrakisphosphate) are internally perfused into the cell, although $IP_3$ alone may cause a small transient activation. They attribute the activation of $K_{Ca}$ current to an increase in $[Ca^{2+}]_i$ due to the action of $IP_3$ and $IP_4$ on intracellular $Ca^{2+}$ stores. It would be interesting to test whether $IP_3/IP_4$ may activate directly $K_{Ca}$ channels of lacrimal acinar cells.

### *Adrenergic Agents*

"Maxi" $K_{Ca}$ channels are modulated by adrenergic agents, as suggested by the initial work in neurons (Madison and Nicoll, 1982; Haas and Konnerth, 1983). $\beta$-Adrenergic stimulation of $K_{Ca}$ channels has been demonstrated in aortic (Sadoshima *et al.*, 1988), tracheal (Kume *et al.*, 1989), and uterine smooth muscle (Toro *et al.*, 1990a). In aortic and tracheal smooth muscle this activation has been studied in both cell-attached and inside-out patches. Experiments in cell-attached patches demonstrated that isoproterenol (0.2–10 $\mu$M) in the perfusion solution, and not in the pipette, enhanced $K_{Ca}$ channel activity (Sadoshima *et al.*, 1988; Kume *et al.*, 1989), while the $\beta$-antagonist propranolol (1 $\mu$M) suppressed the previously activated $K_{Ca}$ channels. These experiments demonstrated that isoproterenol activated $K_{Ca}$ channels by interacting with $\beta$-receptors. They also prompted the authors (Sadoshima *et al.*, 1988; Kume *et al.*, 1989) to search for a mechanism of activation involving a second messenger and not a direct action of isoproterenol on the channel. These studies showed that $K_{Ca}$ channels could also be activated by forskolin (10 $\mu$M) or dibutyryl cAMP (100 $\mu$M) in the absence

of agonist (Sadoshima *et al.*, 1988); and by the inhibitor of protein phosphatases (okadaic acid, 10 $\mu$M) in the absence or in the presence of isoproterenol (Kume *et al.*, 1989). These results were indicative of a cAMP-dependent phosphorylation mechanism producing activation of $K_{Ca}$ channels (Ewald *et al.*, 1985) and of the presence of an endogenous phosphatase. In fact, in both cases (aortic and tracheal smooth muscles) intracellular application of protein kinase A (in inside-out patches) could mimic the stimulatory effect of externally applied isoproterenol, indicating that $K_{Ca}$ channels or a closely related "modulator" were being phosphorylated. Contrary to the findings of Sikdar *et al.* (1989) which suggested that protein kinase activity was present in the isolated patches (see GnRH in this section), the experiments of Kume *et al.* (1989) indicated that kinase activity was only present in the cytosol. The latter conclusion was drawn from experiments in inside-out patches. In them, okadaic acid (protein phosphatase inhibitor) could increase $K_{Ca}$ channel activity in the presence of protein kinase A, but not when protein kinase A was absent from the perfusion solution. These results also indicated the presence of an endogenous protein phosphatase that was membrane bound. Evidently, these differences may be due to the cell type under study, but may also reflect some difficulties in the study of membrane patches. For example, the size of the patch delimiting the number of functional constituents and the possibility of intracellular components remaining within the patch are factors that may influence the results.

To try to circumvent some of these caveats, we have used a different experimental approach. Reconstitution of channels into bilayers has shown to be a reliable method to study $K_{Ca}$ channel regulatory properties. Ewald *et al.* (1985) demonstrated phosphorylation and activation of neuronal $K_{Ca}$ channels (or closely associated "modulator") reconstituted into lipid bilayers on the tip of a patch pipette. Instead, we have used the more commonly used procedure of forming the bilayer across 200 $\mu$m apertures of delrin cups (wall thickness of about 150 $\mu$m) (Miller and Racker, 1976; Latorre *et al.*, 1982; Hamilton *et al.*, 1989). Using this system, we observed that reconstituted $K_{Ca}$ channels from uterine smooth muscle may be activated by "intracellular" GTP or GTP$\gamma$S only in the presence of $Mg^{2+}$ (Ramos-Franco, *et al.*, 1989; Toro *et al.*, 1990a). This result strongly suggested the presence of a G-protein mediated mechanism (Gilman, 1987; Birnbaumer *et al.*, 1987; Brown and Birnbaumer, 1988). To discard the remote possibility that we had in our recording system contaminant ATP, cAMP, and protein kinase A, we tested activation of $K_{Ca}$ channels in the presence of AMP–PNP (adenylyl-imidodiphosphate, a nonphosphorylating analog of ATP). Under these conditions activation of $K_{Ca}$ channels by GTP and $Mg^{2+}$ persisted, indicating that activation of $K_{Ca}$ channels was not mediated by protein phosphorylation. In addition, we demonstrated that this activity could be potentiated by

"extracellular" norepinephrine or the $\beta$-agonist isoproterenol (1–20 $\mu$M) and partially inhibited by the $\beta$-antagonist propranolol (100 $\mu$M, extracellular) or by GDP$\beta$S ($\approx$ 400 $\mu$M, intracellular; a nonhydrolyzable analog of GDP that inhibits G-proteins) (Eckstein *et al.*, 1979). These experiments suggested to us that myometrium possess $\beta$-adrenergic receptors coupled to a GTP-dependent protein that may directly gate $K_{Ca}$ channels. Furthermore, due to the nature of the experimental procedure, it is very likely that the $\beta$-receptor, the G-protein involved, and the $K_{Ca}$ channel form a stable molecular complex that is incorporated as such in the bilayer. Otherwise, we would have expected that unrestrained components would be easily diffusible in the large lipid environment and therefore, we would not be able to detect any activation due to GTP and $Mg^{2+}$. An alternative explanation, though, may be the possibility that $K_{Ca}$ channels are themselves GTP-dependent proteins and have a binding site for $\beta$-agonists. To our knowledge, this possibility is remote and may only be explored when purified or cloned $K_{Ca}$ channels are available.

How are myometrial $K_{Ca}$ channels activated by the putative G-protein? Activation involves a shift toward more negative potentials of the voltage-dependent activation curve (Ramos-Franco *et al.*, 1990) (Fig. 1), makes myometrial $K_{Ca}$ channels behave as if they had a higher $Ca^{2+}$ sensitivity (Toro *et al.*, 1990a), and alters its kinetics by primarily diminishing the value and proportion of long closings (Toro *et al.*, 1990a). Similar changes in $Ca^{2+}$ sensitivity and kinetics were observed in aortic smooth muscle upon phosphorylation (Sadoshima *et al.*, 1988) or upon activation with GMP ($Ca^{2+}$ sensitivity; Williams *et al.*, 1988).

Other mechanisms and metabolic pathways may be involved in the regulation of myometrial $K_{Ca}$ channels by $\beta$-adrenergic agents in the intact cell. Our findings are only the beginning of a long list of metabolites and mechanisms that may be explored in the bilayer system.

### *Angiotensin II (ANG II)*

We have recently shown that $K_{Ca}$ channels ($\approx$ 250 pS) from coronary smooth muscle incorporated into bilayers are inhibited by ANG II. This inhibition is observed after application of the vasoconstrictor peptide to the extracellular site of the channel ($K_{1/2}$ = 58 nM) (Toro *et al.*, 1990b). ANG II diminished the mean $P_o$ of the channel. The minimum number of sites involved in the ANG II–$K_{Ca}$ channel interaction is 2 (Hill coefficient obtained from the dose–response curve). ANG II affected the open and closed states of $K_{Ca}$ channels, and added a new much slower closed state (Fig. 2). Since the experiments were performed in the absence of GTP, $Mg^{2+}$ or ATP, inhibition mediated by an endogenous G-protein or an endogenous protein kinase were unlikely. Therefore, we concluded the ANG II may directly regulate $K_{Ca}$

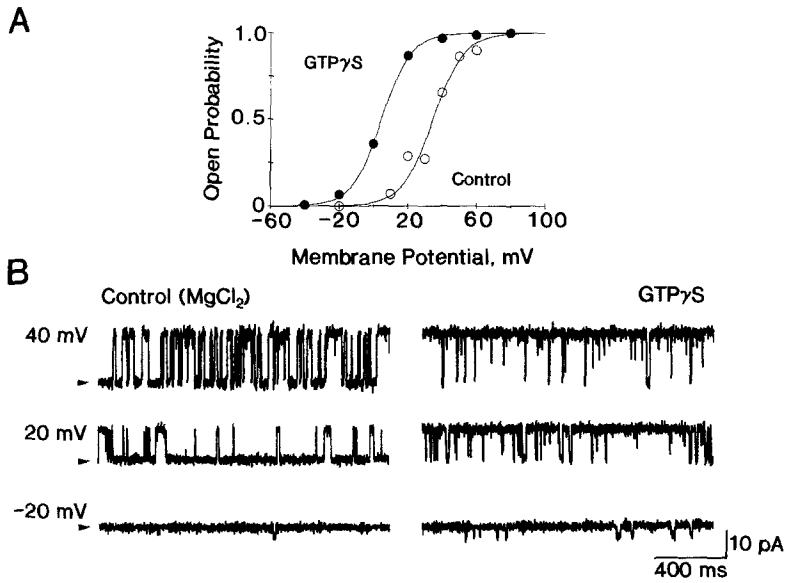


**Fig. 1.** GTPγS increases the voltage sensitivity of $K_{Ca}$ channels from myometrium incorporated into lipid bilayers. A: Open probability vs. membrane potential relationships before (open circles) and after stimulation with "intracellular" GTPγS (closed circles). Experiments were performed in the presence of 1 mM $MgCl_2$. Data were fitted to Boltzmann distributions (continuous lines): $P_o = 1/\{1 + \exp[V_{1/2} - V)/k]\}$, where $P_o$ = open probability, $V_{1/2}$ = half activation voltage, $V$ = applied voltage, and $k$ = slope factor = $RT/zF$ ($z$ is effective valence and $R$, $T$, $F$ have their usual meanings). Fitted values are: (1) Control, $V_{1/2} = 35$ mV, $k = 10$ mV; (2) "G-protein activated" (after 100 μM GTPγS), $V_{1/2} = 5$ mV, $k = 9$ mV. B: $K_{Ca}$ channel activity before and after GTPγS stimulation at different holding potentials. At the same $[Ca^{2+}]_i$ and voltages, the probability of opening is significantly larger in the presence of 100 μM GTPγS. Arrows mark the closed state. Ramos-Franco *et al.*, 1990; J. Ramos-Franco, L. Toro, and E. Stefani, unpublished observations.

channels, being one of the mechanisms by which this peptide produces depolarization and contraction of coronary smooth muscle. Other mechanisms of action of ANG II on $K_{Ca}$ channels have to be explored since it is known that binding of ANG II to its receptor on the cell membrane produces $IP_3$ and diacylglycerol via a G-protein mediated activation of phospholipase C (Bingham, 1986; Lazdunski, 1988).

### *Adenosine and Atrial Natriuretic Factor (ANF)*

$K_{Ca}$ channels (266 pS) from aortic smooth muscle are activated by externally applied adenosine (10 μM) and ANF (100 nM) (Williams *et al.*, 1988). Since the action of adenosine and ANF on vascular smooth muscle produces an increase in intracellualr cGMP, the authors tested the role of this cyclic nucleotide in the activation of $K_{Ca}$ channels. Experiments with cell-attached patches showed that 1 mM dibutyryl cGMP (a permeant analog of

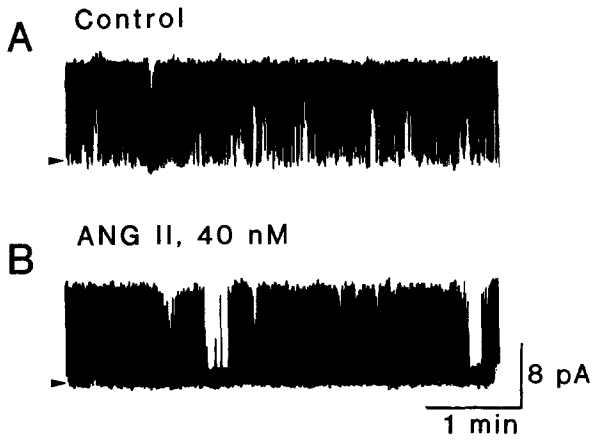


**Fig. 2.** ANG II inhibits $K_{Ca}$ channels from coronary smooth muscle incorporated into lipid bilayers. A: Control traces at +20 mV ($V_{internal}$ = −20 mV). B: After addition of ANG II (40 nM) to the "extracellular" side of the channel. Note the appearance of long closings (≈ 2 s). Arrows mark the closed state.

cGMP) could mimic the activation produced by adenosine and ANF. However, experiments with inside-out patches demonstrated that intracellular cGMP (100 μM–1 mM) activated the channels in a limited fashion and not as expected. In contrast, 1–100 μM GMP could readily activate $K_{Ca}$ channels (see Nucleotide section). These studies suggested to the authors that in the intact cell, GMP was the metabolite responsible for the activation of $K_{Ca}$ channels and not cGMP. Perhaps an hypothetical metabolite route would be: binding of adenosine or ANF to a membrane receptor → activation of a G protein? → activation of guanylate cyclase → ↑$[cGMP]_i$ → cGMP breakdown by a phosphodiesterase → ↑$[GMP]_i$ → activation of $K_{Ca}$ channels.

## Lipids and Metabolites

### *Arachidonic Acid and Fatty Acids*

Large-conductance $K_{Ca}$ channels from pulmonary artery (Ordway *et al.*, 1989) and aortic smooth muscle (Katz *et al.*, 1990) are activated by 50 nM to 20 μM arachidonic acid and by fatty acids like linoelaidic acid (20–40 μM) and myristic acid (20 μM). Since the latter are not substrates of cyclooxygenase nor of lipoxygenase enzymes, the authors have concluded that fatty acids may directly modulate $K_{Ca}$ channels (Ordway *et al.*, 1989) or physically perturb the lipid environment of the channel (Katz *et al.*, 1990). To our knowledge, there is no evidence that metabolites of the arachidonic acid cascade (cyclooxygenase or lipoxygenase products) may regulate $K_{Ca}$ channels,

as has been observed for the muscarinic $K^+$ channel (Kurachi *et al.*, 1989; Kim *et al.*, 1989). Since arachidonic acid may be produced after ligand-receptor activation of phospholipase $A_2$ through a $G_{pla}$-protein mediated mechanism, it would be interesting to investigate if this G-protein plays a regulatory role on $K_{Ca}$ channels.

### *Cholesterol*

The kinetics of $K_{Ca}$ channels (300 pS) from aortic smooth muscle may be altered by the content of cholesterol in the cell membrane (Bolotina *et al.*, 1989). Fluorescence polarization studies showed that deprivation of cholesterol from native membranes increased membrane fluidity. Under these conditions, $K_{Ca}$ channels had an increased $P_o$. The increase in $P_o$ was primarily due to a decrease of the slow closings of the channel. It was concluded that membrane fluidity may affect the kinetic behavior of $K_{Ca}$ channels.

## Nucleotides

### *Adenine Nucleotides*

$K_{Ca}$ channels (40–85 pS, outward rectifying) from unfertilized hamster oocytes are activated by ADP (Yoshida *et al.*, 1990). ADP (100 $\mu$M) applied to the internal solution of an inside-out patch caused the $P_o$ to increase. The increase in $P_o$ was accompanied by a change of the voltage dependence of the channel ($P_o$ became voltage independent). To our knowledge this is the only known metabolite that produces a change in voltage dependence of $K_{Ca}$ channels.

In contrast, "maxi" $K_{Ca}$ channels from aortic smooth muscle were insensitive to internal cAMP (Sadoshima *et al.*, 1988), AMP, ADP, and ATP (Williams *et al.*, 1988).

### *Guanine Nucleotides*

In aortic $K_{Ca}$ channels (266 pS), GMP (100 $\mu$M) has been proposed as the metabolite of a reaction cascade (initiated by adenosine, ANF, or nitroprusside) responsible for the activation of $K_{Ca}$ channels (Williams *et al.*, 1988). GMP affected both the voltage and calcium sensitivities of $K_{Ca}$ channels. GMP shifted the voltage-dependent activation curve toward more negative potentials and the calcium-dependent activation curve toward lower $[Ca^{2+}]_i$. cGMP, GDP, and GTP potentiated $K_{Ca}$ channel activity, but at a higher concentration (500 $\mu$M).

## Conclusions

Evidently, what this review shows is that $K_{ca}$ channels are a family of channels with a high metabolic regulation. Depending on the tissue and modulator, $K_{Ca}$ channels may be involved in a wide spectrum of cellular functions which may be summarized in two actions: excitatory or inhibitory. Furthermore, their response may be cyclical, not only originated by transient changes in intracellular $Ca^{2+}$ but by other intracellular modulators like GMP (Williams *et al.*, 1988). Taking into account not only their regulation by internal $Ca^{2+}$, $K_{Ca}$ channels are now thought to be involved in functions as opposite as smooth muscle contraction and relaxation. Since modulators may affect both their voltage and calcium sensitivities, and in the case of oocytes its voltage dependence, the usual assumption that their main physiological function was gained after a rise in $[Ca^{2+}]_i$ had taken place has now to be reconsidered.

## Acknowledgments

This work was supported by Grant-in-Aid 88G-189 from the American Heart Association, Texas Affiliate (L.T.), and by grants HD-25616 and AR-38970 from the National Institutes of Health (E.S.).

## References

Bean, B. P. (1989). *Annu. Rev. Physiol.* **51**, 367–384.
Benham, C. D., and Bolton, T. B. (1986). *J. Physiol.* **381**, 385–406.
Bingham, S. J. (1986). *Am. J. Physiol.* **250**, F759–F769.
Birnbaumer, L., Codina, J., Mattera, R., Yatani, A., Scherer, N., Toro, M.-J., and Brown, A. M. (1987). *Kidney Int.* **32**, S14–S37.
Birnbaumer, L., VanDongen, A. M. J., Codina, J., Yatani, A., Mattera, R., Graf, R., and Brown, A. M. (1989). In *Secretion and Its Control* (Armstrong, C. M. and Oxford, G. S., eds.), Rockefeller University Press, New York, pp. 17–54.
Bolotina, V., Omelyanenko, V., Heyes, B., Ryan, U., and Bregestovski, P. (1989). *Pflügers Arch.* **415**, 262–268.
Brown, A. M., and Birnbaumer, L. (1988). *Am. J. Physiol.* **254**, H401–H410.
Brown, A. M., and Birnbaumer, L. (1990). *Annu. Rev. Physiol.* **52**, 197–213.
Carl, A., and Sanders, K. M. (1989). *Am. J. Physiol.* **257**, C470–C480.
Cole, W. C., and Sanders, K. M. (1989). *Am. J. Physiol.* **257**, C596–C600.
Cole, W. C., Carl, A., and Sanders, K. M. (1989). *Am. J. Physiol.* **257**, C481–C487.
Eckstein, F., Cassel, D., Levkovitz, H., Lowe, M., and Selinger, Z. (1979). *J. Biol. Chem.* **254**, 9829–9834.
Edwards, K. M., Jackson, B. A., and Dousa, T. P. (1980). *Am. J. Physiol.* **238**, F269–F278.
Ewald, D. A., Williams, A., and Levitan, I. B. (1985). *Nature (London)* **315**, 503–506.
Gilman, A. G. (1987). *Annu. Rev. Biochem.* **56**, 615–649.
Guggino, S. E., Suarez-Isla, B. A., Guggino, W. B., and Sacktor, B. (1985). *Am. J. Physiol.* **249**, F448–F455.

Haas, H. L., and Konnerth, A. (1983). *Nature (London)* **302**, 432–434.
Hamilton, S. L., Mejia-Alvarez, R., Fill, M., Hawkes, M. J., Brush, K. L., Schilling, W. P., and Stefani, E. (1989). *Anal. Biochem.* **183**, 31–41.
Higashida, H., and Brown, D. A. (1986). *Nature (London)* **323**, 333–335.
Katz, G., Roy-Contancin, L., Bale, T., and Reuben, J. P. (1990). *Biophys. J.* **57**, 506a.
Kim, D., Lewis, D. L., Graziadei, L., Neer, E. J., Bar-Sagi, D., and Clapham, D. E. (1989). *Nature (London)* **337**, 557–560.
Kume, H., Takai, A., Tokumo, H., and Tomita, T. (1989). *Nature (London)* **341**, 152–154.
Kurachi, Y., Ito, H., Sugimoto, T., Shimizu, T., Miki, I., and Ui, M. (1989). *Nature (London)* **337**, 555–557.
Latorre, R., Vergara, C., and Hidalgo, C. (1982). *Proc. Natl. Acad. Sci. USA* **79**, 805–809.
Latorre, R., Oberhauser, A., Labarca, P., and Alvarez, O. (1989). *Annu. Rev. Physiol.* **51**, 385–399.
Lazdunski, M. (1988). *Am. J. Med.* **84**, Suppl. 1B, 3–9.
Madison, D. V., and Nicoll, R. A. (1982). *Nature (London)* **299**, 636–638.
Mason, W. T., and Waring, D. W. (1986). *Neuroendocrinology* **43**, 205–219.
Mayer, E. A., Loo, D. D. F., Kodner, A., and Narashima Reddy, S. (1989). *Am. J. Physiol.* **257**, G887–897.
Mayer, E. A., Loo, D. D. F., Snape, W. J., Jr, and Sachs, G. (1990). *J. Physiol.* **420**, 47–71.
Miller, C., and Racker, E. (1976). *J. Membr. Biol.* **30**, 283–300.
Morris, A. P., Gallacher, D. V., Irvine, R. F., and Petersen, O. H. (1987). *Nature (London)* **330**, 653–655.
Neliat, G., Masson, F., and Gargouil, Y. M. (1989). *Pflügers Arch.* **414**, S186–S187.
Ordway, R. W., Clapp, L. H., Gurney, A. M., Singer, J. J., and Walsh, J. V., Jr. (1989). *J. Gen. Physiol.* **94**, 37a.
Ramos-Franco, J., Toro, L., and Stefani, E. (1989). *Biophys. J.* **55**, 536a.
Ramos-Franco, J., Toro, L., and Stefani, E. (1990). *Biophys. J.* **57**, 112a.
Sadoshima, J.-I., Akaike, N., Kanaide, H., and Nakamura, M. (1988). *Am. J. Physiol.* **255**, H754–H759.
Sauvé, R., Simoneau, C., Parent, L., Monette, R., and Roy, G. (1987). *J. Membr. Biol.* **96**, 199–208.
Shangold, G. A., Murphy, S. N., and Miller, R. J. (1988). *Proc. Natl. Acad. Sci. USA* **85**, 6566–6570.
Sikdar, S. K., McIntosh, R. P., and Mason, W. T. (1989). *Brain Res.* **496**, 113–123.
Toro, L., Ramos-Franco, J., and Stefani, E. (1990a). *J. Gen. Physiol.* **96**, 373–394.
Toro, L., Amador, M., and Stefani, E. (1990b). *Am. J. Physiol.* **258**, H912–H915.
Turgeon, J. L., and Waring, D. W. (1986). *Am. J. Physiol.* **250**, E62–E68.
Williams, D. L., Jr., Katz, G. M., Roy-Contancin, L., and Reuben, J. P. (1988). *Proc. Natl. Acad. Sci. USA* **85**, 9360–9364.
Yoshida, S., Plant, S., McNiven, A. I., and House, C. R. (1990). *Pflügers Arch.* **415**, 516–518.