ISSN 1669-5402 (Print) ISSN 1669-5410 (Online)

Physiological Mini-Reviews

Edited by the Argentine Physiological Society.

Vol. 1, N° 8, March 2006.

http://www.mini.reviews.safisiol.org.ar

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[ISSN 1669-5402 (Print); ISSN 1669-5410 (Online)]

Edited by the **Argentine Physiological Society**

Journal address: Sociedad Argentina de Fisiología, Universidad Favaloro, Solís 453 (1078),
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Role of sarcolemmal ATP-dependent K^+ channels in cardiac ischemic injury.

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Introduction

In the normoperfused heart, Ca^{2+} enters the myocardial cell through voltage-dependent Ca^{2+} channels and is extruded through the sarcolemmal Ca^{2+} pump and the Na^+/Ca^{2+} exchanger. During ischemia and early reperfusion, however, sarcoplasmic reticulum reuptake and sarcolemmal Ca^{2+} pump activities are impaired due to decreased ATP. In addition, owing to defective Na^+-K^+ pump performance and low pH favoring Na^+ entry in exchange for H^+ output through the Na^+/H^+ exchanger, the Na^+/Ca^{2+} exchanger operates in its reverse mode incorporating Ca^{2+} to diminish increased intracellular Na^+. Thus, the resulting impaired Ca^{2+} balance produces Ca^{2+} overload and myocardial injury (14) probably by activation of Ca^{2+}-dependent proteases which can partially destroy contractile proteins leading to decreased responsiveness of contractile filaments to Ca^{2+} (18). This chain of ischemic events is in part counteracted by activation of ATP-dependent K^+ (KATP) channels which by allowing earlier passive K^+ output, shortens action potential duration and reduces the time during which Ca^{2+} can enter the cell.

KATP channel structure

Originally discovered by Noma (21) in the heart, KATP channels have been found in other tissues including the brain, skeletal and smooth muscle, renal and tracheal epithelium, smooth muscle of the urinary tract and pancreas (17). KATP channels are members of the inwardly rectifying K^+ channel superfamily (Kir), formed by the fusion of four specific Kir subunits, called Kir 6.x and four sulfonylurea receptors (SUR). At least two inwardly rectifying subunits, Kir6.1 and Kir6.2 and three sulfonylurea receptors, SUR1, SUR2A and SUR2B have been identified, forming channels in different tissues (12). Specifically, the combination of Kir6.2 and SUR1 is found in pancreatic cells while Kir6.2 and SUR2A form the channels in cardiac and skeletal muscle cells (15).

Two types of KATP channels have been identified: sarcolemmal and mitochondrial. Sarcolemmal KATP channels are thought to serve as a link between cell metabolism and either secretory activity in the pancreas and brain, or electro-mechanical coupling in muscle cells. On the other hand, mitochondrial KATP channels, which are found in the inner membrane of the mitochondrion mediate K^+ influx into the mitochondrial matrix, regulating mitochondrial volume. This review will focus on sarcolemmal KATP channels.

Mechanism of sarcolemmal KATP channel function

The main characteristic of both mitochondrial and KATP channels is that their opening is modulated by the intracellular ATP concentration (**Fig.1**). Thus, in normal oxygenated conditions and high ATP concentration, KATP channels are closed. As ATP deposits are depleted during ischemia or hypoxia, KATP channels open allowing K^+ efflux with the concomitant action potential phase 3 shortening. This reduces action potential duration (APD) and the time of Ca^{2+} influx through voltage-dependent Ca^{2+} channels, thus

decreasing damage due to Ca^{2+} overload. Simultaneously, the diminished Ca^{2+} entry reduces contractile activity during ischemia preserving ATP sources needed for mechanical recovery at the start of reperfusion when the metabolic mechanisms are not still fully recovered (4, 24). Regulation of KATP channel function is effected through an ATP regulatory site, a phosphorylation subunit and nucleotide diphosphate binding sites. KATP channel opening occurs when diphosphate nucleotides such as ADP block ATP inhibition on channel opening, an action that requires the occupation of a phosphorylation site by inorganic phosphate (PO_4). Binding of endogenous metabolites, such as adenosine, acetylcholine or bradykinin to specific membrane receptors coupled to inhibitory G proteins regulate sarcolemmal KATP channel opening via protein kinase C activation (10, 12, 22). On the other hand, KATP channel openers (pinacidil, nicorandil, cromakalin, etc) enhance channel opening stimulating the ADP inhibitory site or antagonizing the inhibitory effect of ATP.

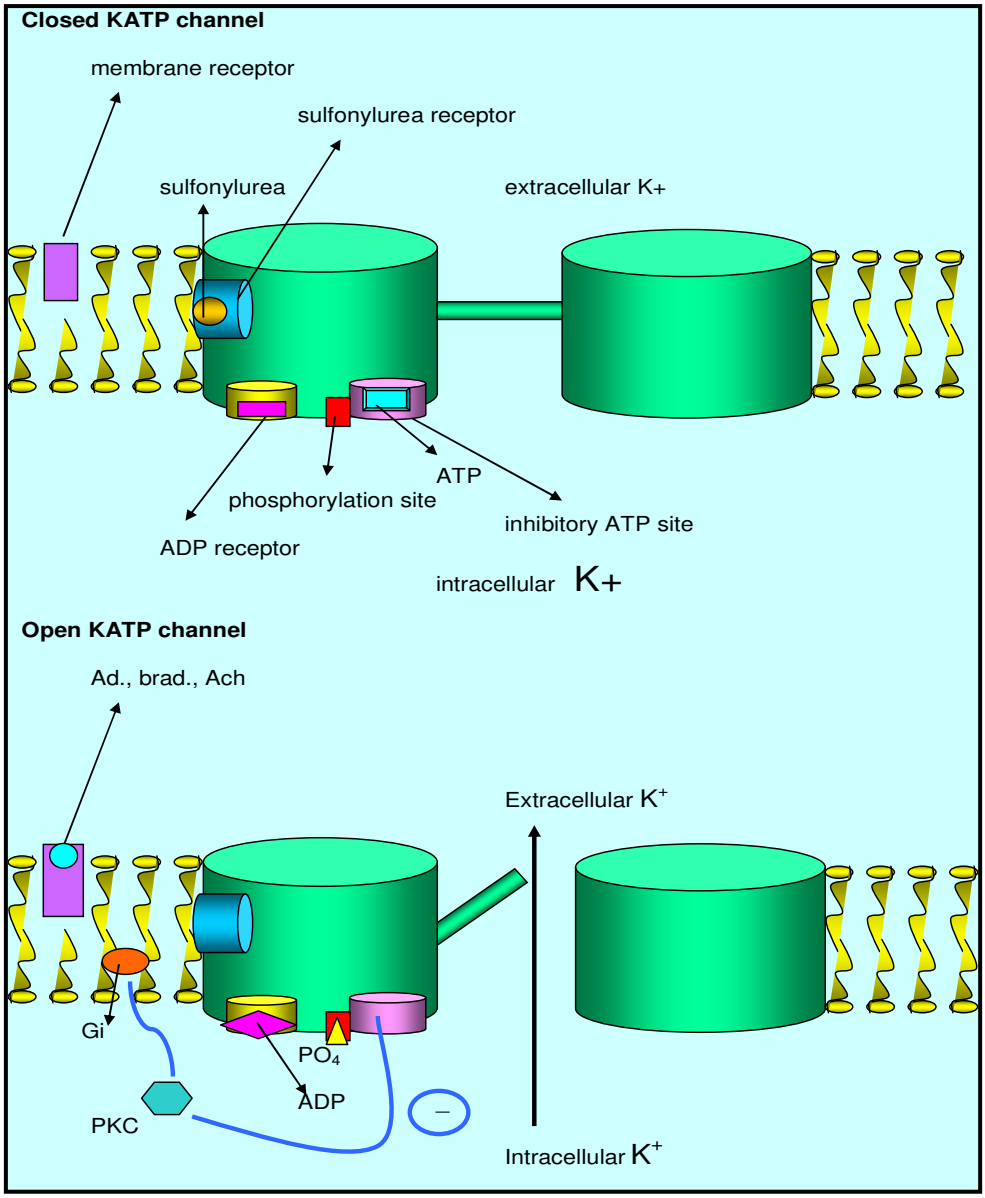

Fig. 1. *Diagram showing regulation of the sarcolemmal KATP channel. ATP binding to the ATP inhibitory site or drug binding to the sulfonylurea receptor reduces the open state probability of the channel. In contrast, ATP decrease during ischemia and ADP binding to a specific receptor induce channel opening, an action that requires occupation of a phosphorylation site with PO_4. Release of adenosine (Ad), bradykinin*

(brad.) and acetylcholine (Ach) among other metabolites activate G_i membrane proteins and protein kinase C antagonizing the ATP inhibitory site.

Association between action potential shortening and Ca^{2+} overload

Decrease of myocardial injury with interventions reducing Ca^{2+} influx at reperfusion and greater mechanical recovery obtained with KATP channel openers (1, 2, 13) suggested an association between ischemia-induced APD shortening by sarcolemmal KATP opening and reduced Ca^{2+} influx. This hypothesis which was first reported in several isolated myocyte studies (16, 29) was then studied in our laboratory in a conscious animal model. Because in a previous work we had found that the KATP channel blocker glibenclamide, a sulfonylurea which acts as a non-specific KATP channel blocker, inhibited monophasic APD shortening during ischemia (**Fig.2**) and increased postischemic stunning (20), we postulated that augmented myocardial dysfunction with glibenclamide was produced by enhanced Ca^{2+} influx due to APD prolongation, and that consequently a decrease of Ca^{2+} entry by means of a sarcolemmal L-channel inhibitor would improve mechanical function. Effectively, the study showed that increased stunning provoked by glibenclamide blockade during a 12 min regional ischemic insult in conscious sheep was partially reverted by diltiazem (19), an L-channel blocker whose action is not affected by glibenclamide (23) (**Fig.3**).

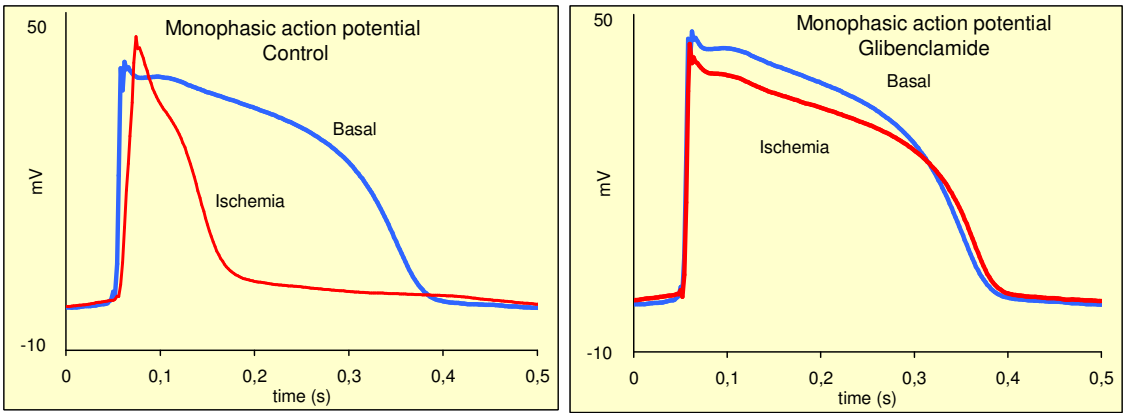

Fig. 2. *Time course of epicardial monophasic action potential in open chest sheep. In control conditions, a 12 min ischemic period decreased MAPD by 50% due to KATP channel opening, an effect that was completely blocked by glibenclamide.*

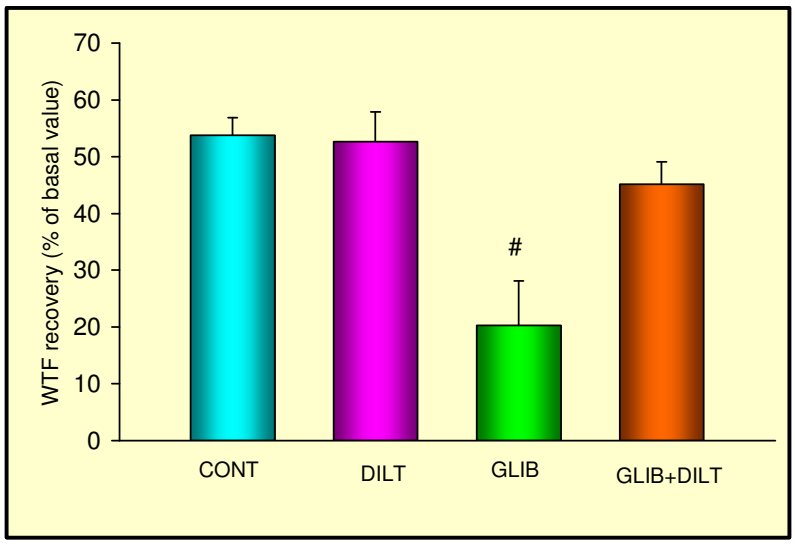

Fig. 3. *Mean percent recovery of regional wall thickening fraction (WTF= 100 * end systolic wall thickness-end diastolic wall thickness)/end diastolic wall thickness) over the 2 h reperfusion period following a 12 min ischemic period. Diltiazem (DILT) administration did not differ from control (CONT). Glibenclamide (GLIB), 0.4 mg/kg, reduced postischemic mechanical WTF. This was almost completely recovered with the addition of diltiazem (GLIB + DILT). Mean ± SE;# p< 0.01 GLIB vs CONT, DILT or GLIB + DILT, ANOVA followed by Scheffé.*

However, the incomplete functional recovery of the glibenclamide + diltiazem group indicated that Ca^{2+} entry was not only effected through sarcolemmal Ca^{2+} channels but posibly, also by the reverse mode of the Na^{+}/Ca^{2+} exchanger.

Opening of sarcolemmal KATP channels accompanied by APD shortening has been also associated to protection against infarction (27, 30) and against arrhythmia incidence (9). Supporting these findings, we demonstrated in conscious sheep that inhibition of KATP channels with glibenclamide aggravated postischemic arrhythmic episodes (5) probably due to Ca^{2+} overload (3).

Role of KATP channels in preconditioning protection

Because of their involvement in ischemic events, KATP channels were almost immediately associated with the protection afforded by myocardial preconditioning, a phenomenon whereby brief periods of ischemia-reperfusion trigger a chain of events that culminate in the protection of the myocardium submitted to a subsequent ischemic period, reducing infarct size, reperfusion-induced arrhythmias and postischemic dysfunction. There are two preconditioning time frames: early preconditioning which protects within 2-3 h after the preconditioning stimulus and late preconditioning which reappears 24 h later and may last up to 3 days. Gross and Auchampach (11) were the first to demonstrate KATP channel participation in early preconditioning protection against infarction, evidenced by the reduction in necrosis obtained with the KATP channel opener aprikalim and its inhibition by glibenclamide, suggesting a role of KATP channel as effectors of the protective mechanism. Contrary to these results, no role of KATP channels in late preconditioning protection against stunning in conscious rabbits (26) or sheep (20) disclaimed preconditioning protection through reduction in Ca^{2+} overload. Moreover, studies showing protection with diazoxide, a KATP channel opener that did no shorten APD tipped the balance in favor of mitochondrial rather than sarcolemmal channels as end-effectors of preconditioning (12, 22). However, further studies in other animal models with different endpoints indicated that a conclusive demonstration of the relative importance of each type of KATP channel in preconditioning protection has not yet been clearly established. In isolated rabbit hearts Toyoda et al (28) presented evidence suggesting that reduction in infarct size would be modulated by mitochondrial KATP channels, while mechanical recovery would be governed by sarcolemmal KATP channels, and in knockout mice deficient for sarcolemmal KATP channels with preservation of mitochondrial ones, no protection against infarct size supports an important role of sarcolemmal KATP channels in preconditioning protection (25). Furthermore, even though sarcolemmal KATP channels do not seem to participate in ischemic late preconditioning against arrhythmia in conscious sheep (20), a recent study showed that early preconditioning defense against ventricular arrhythmias induced by low-flow ischemia involved sarcolemmal but not mitochondrial KATP channels (8).

KATP channel alteration in diabetic hearts

Findings of KATP channel dysfunction in pathologic hearts support the concept relating KATP channel activation and APD shortening to decreased Ca^{2+} overload. In this sense, greater KATP lengthening of APD during early reperfusion in conscious diabetic sheep (**Fig.4A**) might explain lack of functional mechanical recovery from stunning and absence of early and late preconditioning protection, and similarly, KATP channel alterations would substantiate the greater deleterious effect of glibenclamide on postischemic stunning in this disease (**Fig.4B**) (6). Insufficient insulin might be responsible for the impaired KATP channel behavior in diabetes, since sheep treated with insulin during two weeks almost completely reverted APD to that of nondiabetic animals, restoring early and late preconditioning protection against stunning (6, 7).

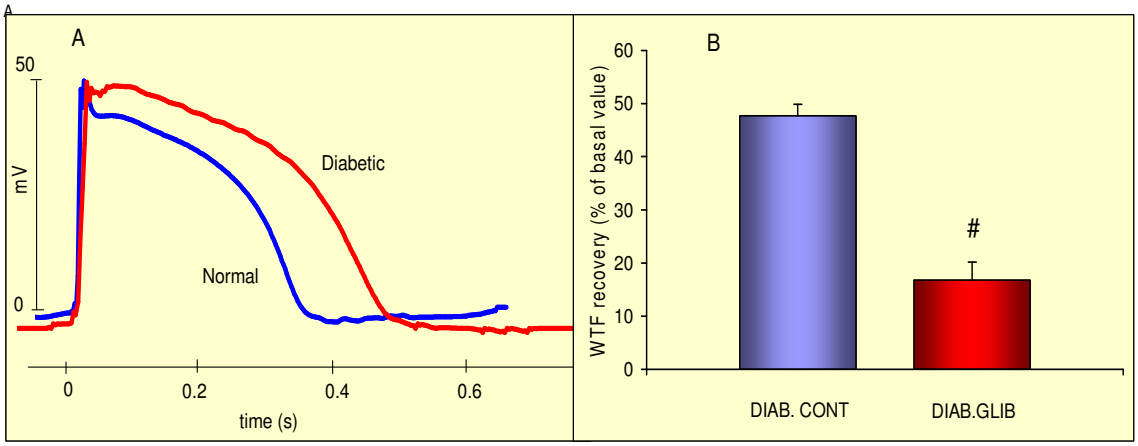

Fig. 4. *A: Monophasic action potential in normal (nondiabetic) and diabetic sheep. Diabetes increased APD by 32%. B: Mean percent recovery of regional wall thickening fraction (WTH) over the 2 h reperfusion period following a 12 min ischemic period in conscious alloxan diabetic sheep without (DIAB CONT) and with glibenclamide (DIAB. GLIB). Glibenclamide at a much lower dose (0.1 instead of 0.4 mg/kg) than that used in nondiabetic animals produced a similar reduction in recovery (see Fig. 3), indicating greater sulfonylurea sensitivity in this disease. Mean ±SE; # p<0.0, t test.*

In conclusion, opening of KATP channels during ischemia and early reperfusion seems to be an inherent protective mechanism of the heart to reduce myocardial injury, and channel impairment occurring in certain pathologic conditions such as diabetes, might explain in part the increased deleterious effect of ischemia-reperfusion events in patients treated with sulfonylureas to control insulin release through the pancreatic KATP channel.

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XXII CONGRESO LATINOAMERICANO Y 1ER IBERO-AMERICANO DE CIENCIAS FISIOLÓGICAS

Organizado por la Sociedad Argentina de Fisiología, por decisión de la Asociación Latinoamericana de Ciencias Fisiológicas (ALACF) y con el auspicio de la Sociedad Española de Ciencias Fisiológicas
Buenos Aires, 4 al 7 de noviembre de 2006

Este año tendrá lugar en Buenos Aires el XXII Congreso de la Asociación Latinoamericana de Ciencias Fisiológicas (ALACF). Esta reunión congregará a científicos originarios de América Latina trabajando en sus países de origen, en Estados Unidos, en Europa y alrededor del mundo. Fisiólogos no latinoamericanos de primer nivel son también regularmente invitados. Esta vez la Sociedad Española de Ciencias Fisiológicas se asocia al evento, dándole especial interés y relevancia.

El objetivo central del Congreso es dar, a los fisiólogos trabajando y viviendo en Latinoamérica, la posibilidad de entrar en contacto con referentes en su campo de trabajo. Esto será especialmente cierto esta vez para aquellos radicados en el Cono Sur del continente (Bolivia, Brasil, Chile, Paraguay, Uruguay y Argentina).

Estructura del Congreso:

El Congreso presentará Conferencias Plenarias, Mini-Conferencias, Simposios y presentaciones de Carteles (Posters). Las lenguas oficiales son el español, portugués e inglés. Se llevará a cabo en las sedes de la Universidad Favaloro y del Centro Asturiano de Buenos Aires.

La estructura de base del Programa será la siguiente:

Horario	**Sábado**	**Domingo**	**Lunes**	**Martes**
08.30-10.30		Simposios	Simposios	Simposios
10.30-11.00		Pausa Café	Pausa Café	Pausa Café
11.00-12.00	Inscripción	Conferencias	Conferencias	Conferencias
12.00-13.00	Almuerzo	Almuerzo	Almuerzo	Almuerzo
13.00-15.30	Inscripción	Pres. Carteles	Pres. Carteles	Pres. Carteles
15.30-17.30	Simposios	Simposios	Simposios	Simposios
17.30-18.00		Pausa Café	Pausa Café	Pausa Café
18.00-19.00	Conferencias	Conferencias	Conferencias	Conferencias
19.30-21.00	Encuentro (get together)	Asamblea ALACF	Asamblea SAFIS	
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