Inhibition of oxygen consumption in skeletal muscle-derived mitochondria by pinacidil, diazoxide, and glibenclamide, but not by 5-hydroxydecanoate

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Abstract Cell intermediary metabolism and energy production succeeds by means of mitochondria, whose activity is in relation to transmembrane potential and/or free radical production. Adenosine triphosphate (ATP)-dependent potassium channels (K_{ATP}) in several cell types have shown to couple cell metabolism to membrane potential and ATP production. In this study, we explore whether oxygen consumption in isolated skeletal-muscle mitochondria differs in the presence of distinct respiration substrates and whether these changes are affected by K_{ATP}-channel inhibitors such as glibenclamide, 5-Hydroxydecanoate (5-HD), and K_{ATP} channel activators (pinacidil and diazoxide). Results demonstrate a concentration-dependent diminution of respiration rate by glibenclamide (0.5–20 μM), pinacidil (1–50 μM), and diazoxide (50–200 μM), but no significant differences were found when the selective mitochondrial K_{ATP}-channel inhibitor (5-HD, 10–500 μM) was used. These results suggest that these K_{ATP}-channel agonists and antagonists exert an effect on mitochondrial respiration and that they could be acting on mito-K_{ATP} or other respiratory-chain components.

Keywords Pinacidil · Glibenclamide · Muscle mitochondria · K-ATP channel · Oxygen consumption

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Introduction

Mitochondria are essential organelles for cell intermediary metabolism and energy generation via cell respiration. In isolated mitochondria, the respiratory chain consists of a series of electron carriers, the majority of which are integral membrane proteins, capable of accepting and donating electrons (Nicholls and Ferguson 2002). During oxidative phosphorylation, the electrons are conducted through mitochondrial respiratory complexes, and a proton gradient establishes across the inner mitochondria membrane, as the energy source for ATP production succeeds according to the cell's energetic demand.

In the phosphorylation pathway, several points can be pharmacologically manageable via available agents that block respiration at each one of the respiratory chain complexes; some others inhibit ATP synthase, while still others, such as protonophores, short-circuit the electrochemical potential for protons (primarily expressed as a mitochondrial transmembrane potential) and uncouple respiration from phosphorylation. Consequently, systematic dissection of mitochondrial function under several physiological conditions and its respective measurements, provide us information on the behavior of this organelle regarding its trans-membrane potential and redox state (Duchen 1999).

The rate of Adenosine triphosphate/Adenosine biphosphate (ATP/ADP) production also regulates the activity of sarcolemmal and mitochondrial potassium permeability through K_{ATP} channels, because ATP production and metabolism have been related with membrane and redox potential, and to free-radical production (O'Rourke et al. 1994; Hoppeler et al. 2003). Plasmatic membrane K_{ATP} channels couple cell metabolism to membrane excitability (Tricarico et al. 2000; O'Rourke 2000; Debska et al. 2002; Das et al. 2003) and they act as a target for sulphonylureas (Aguilar-Bryan et al. 1995). In mitochondria, K_{ATP} channels (mito-K_{ATP}) are involved in the coupling of redox potential (O'Rourke et al. 1994) and/or in free-radical release (Hoppeler et al. 2003).

On the other side, during muscle contraction, myofibrils consume ATP according to energy demand, but on prolonged exercise, the oxygen demands increase (Brooks et al. 1999) and skeletal-muscle fibers exhibit a progressive decline of performance that largely recovers after a resting time, in a reversible phenomena known as fatigue (Allen et al. 2008; Allen 2009). In these physiological phenomena in the muscle cell, the oxygen concentration decays about 3.1% with respect to the resting condition. Then, mito-K_{ATP} could play an acute role acting in response to hypoxia (Hoppeler et al. 2003). It has been shown that tension development in muscle cells depends on the partial oxygen pressure (Eu et al. 2003) and it might depends on ATP production via modulation of excitation-contraction coupling. In this step, the surrounding mitochondria of the muscle cells are responsible for generating ATP to supply energy demands, exhibiting in their internal membrane permeability transitions (Green and Reed 1998), redox oscillations (O'Rourke et al. 1994), and response to ischemia (Vander Heide et al. 1996).

K_{ATP} channels show structural and pharmacological differences according to their localization, it is, whether they are in the plasmatic membrane or inner mitochondrial membrane. Drugs such as pinacidil appear to activate both types of K_{ATP} channels, but glibenclamide can inhibit them (Paucek et al. 1992; Garlid et al. 1997).

An electrophoretic K^+ influx occurs in two ways: via simple diffusion or through K_{ATP} channels. Activation of mito-K_{ATP} will cause an increase in mitochondrial matrix volume, which in turn could activate the respiratory chain (Halestrap 1989; Grover and Garlid 2000; O'Rourke 2000; Debska et al. 2002; Das et al. 2003). A K^+ cycle was explored by Garlid and Paucek (2001), who demonstrated that K^+ is driven into the matrix by the membrane potential, which is generated by proton-pumping of the electron transport system. Thus, excessive K^+ is removed by the regulated K^+/H^+ antiporter. The regulation of the K^+ fluxes is the manner that volume regulates in the face of the cell's changing its energy requirements (Mironova et al. 2004). In

addition, the transitory permeability pore could be pharmacologically modulated as suggested by Dahlem et al. (2006). Its activation could cause functional changes such as changes in intracellular Ca^{2+} concentration and mitochondrial potential (Nicholls 2005), which contributes to regulate ATP production; for example, induced mitochondrial uncoupling could induce rapid activation of the potassium currents carried through K_{ATP} channels in the presence of pinacidil (Sasaki et al. 2001).

On the other hand, the action of a specific mito-K_{ATP}-channel inhibitor, such as 5-Hydroxydecanoate (5-HD) or specific activators such as diazoxide (Garlid et al. 1997; Sato et al. 1998) could aid in establishing differences in mitochondrial responses to several experimental conditions. Thus, the aim of this work was to explore the effects of specific K_{ATP}-channel activators, such as pinacidil and diazoxide, and the action of inhibitors such as glibenclamide and 5-HD on oxygen consumption in isolated mitochondria derived from chicken skeletal muscle.

Materials and methods

Isolated mitochondria from pectoralis muscle of 3-week-old Arbor Acres chickens were used to explore oxygen consumption in the absence or presence of drugs acting on K_{ATP} channels. The chicks were euthanized prior to dissection of the pectoralis muscle (Huerta and Stefani 1981). Animal maintenance procedures were managed according to Mexican Regulations for Use and Animal Care (NOM-062-ZOO-1999), and the Ethics Committee at our Institution approved the protocol.

Solutions

Ginsborg saline (modified from Huerta and Stefani 1981) was employed for muscle dissection. Its composition (in mM) was as follows: NaCl, 167; KCl, 5; $MgCl_2$, 2; $CaCl_2$, 5; mannitol, 2 g/L. pH was adjusted to 7.4 with Imidazol-Cl (2 mM). For mitochondria isolation, solutions 1 and 2 were used: Solution-1 was (in mM): Sucrose, 100; Trizma base, 50; KCl, 50; EDTA, 5. Solution-2 was (in mM): Sucrose, 250: EGTA 1; Trizma base, 20. pH was adjusted to 7.4 with HCl (Barre et al. 1989). Oximetry solution (in mM) was the following: KCl, 120; KH_2PO_4, 5; Hepes, 3; EGTA, 1; $MgCl_2$, 1, assuring a pH of 7.4 with Trizma base (Barre et al. 1989).

Skeletal-muscle mitochondria isolation

After dissection of chicken´s pectoralis muscle, the latter was weighed and immersed in Ginsborg saline. Mitochondria were isolated by differential centrifugation in a Percoll

gradient as described by Thakar and Hassan (1988) and Sims (1990). In brief, Nagarase (1 µg/mg muscle) was added to extract inter-myofibrillar mitochondria (Talbot et al. 2003, 2004); pre-cooling of the muscle at 4 °C in solution-1 was required, as well as muscle sectioning and homogenizing to ensure enzyme action. The homogenate was centrifuged and placed in a discontinuous Percoll gradient (15, 23, and 40%) at 30,700 *g* for 15 min at 4 °C in a Beckman J6 MY centrifuge. Mitochondria were isolated, diluted 1:4, centrifuged again, and washed at 16,700 *g* in isolation medium to which 0.5% of bovine serum albumin was added, followed by a final centrifugation at 6,900 *g* for 10 min (Calderón-Cortés et al. 2008). The pellet containing mitochondria was resuspended in solution-2 and the mitochondrial protein concentration was measured by the Biuret method (Gornall et al. 1949).

Measurement of oxygen consumption

Once mitochondria were obtained and protein concentration was determined, 1 mg/ml of protein was placed into the oxymeter chamber to measure oxygen consumption polarographically with a Clark-type oxygen sensor placed in a 2-ml closed chamber at 30 °C under continuous stirring in an air-saturated phosphate buffer (50 mM KH_2PO_4). Addition of rotenone (5 µM) and the substrate for complex II, succinate (Hanley et al. 2002), as well as ADP (100 mM), was effected in order to induce state 3 of mitochondrial respiration, in addition to glutamate-malate for complex I (10 mM). A standardized scale at 100% was utilized and dissolved oxygen 800 nat O_2/mL and changes in oxygen consumption were monitored under different experimental protocols.

To explore the effects of the experimental drugs on oxygen consumption, the following range of concentrations was employed: glibenclamide (0.5, 1, 5, 10, and 20 µM); pinacidil (1, 5, 10, and 50 µM); 5-HD (100 and 500 µM), and diazoxide (50 and 200 µM). All of these were diluted in dimethyl sulfoxide (glibenclamide), water (pinacidil and 5-HD), or NaOH 0.1 M (diazoxide) and prepared as stock solutions. All experiments were performed at room temperature (20–22 °C).

Data analysis

Concentration-effect curves were obtained using the appropriate software (Origin ver. 6.0, Origin Lab. Corp., Northampton, MA, USA) and data were adjusted to the Hill equation $\left[I = I_{max} / \left(1 + (EC_{50}/x)^h \right) \right]$. Results are reported as mean ± Standard error of the mean (SEM). Statistical differences of the data were determined with the Student *t* test and were considered statistically significant at $p < 0.05$

Results

Effect of glibenclamide on mitochondrial oxygen consumption

We explored the effects of the K_{ATP} channel blocker Glibenclamide on mitochondrial oxygen consumption in the state-3 from mitochondrial respiration. Oxygen consumption measurements exhibited a dose-dependent inhibition of mitochondrial respiration by glibenclamide. Inhibition of respiration was 40% in the presence of glibenclamide (0.5 µM); further concentrations (1, 5, 10, and 20 µM) inhibited mitochondrial respiration by up to 95% in state 3 (Fig. 1a).

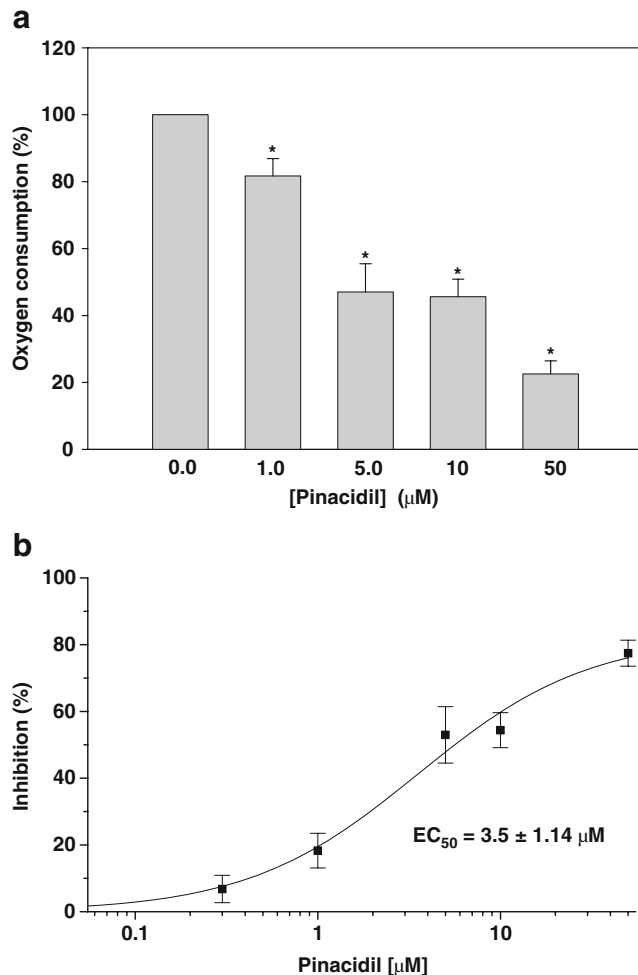Figure 1b shows the dose-response curve for glibenclamide respiration inhibition. Glibenclamide inhibition`s
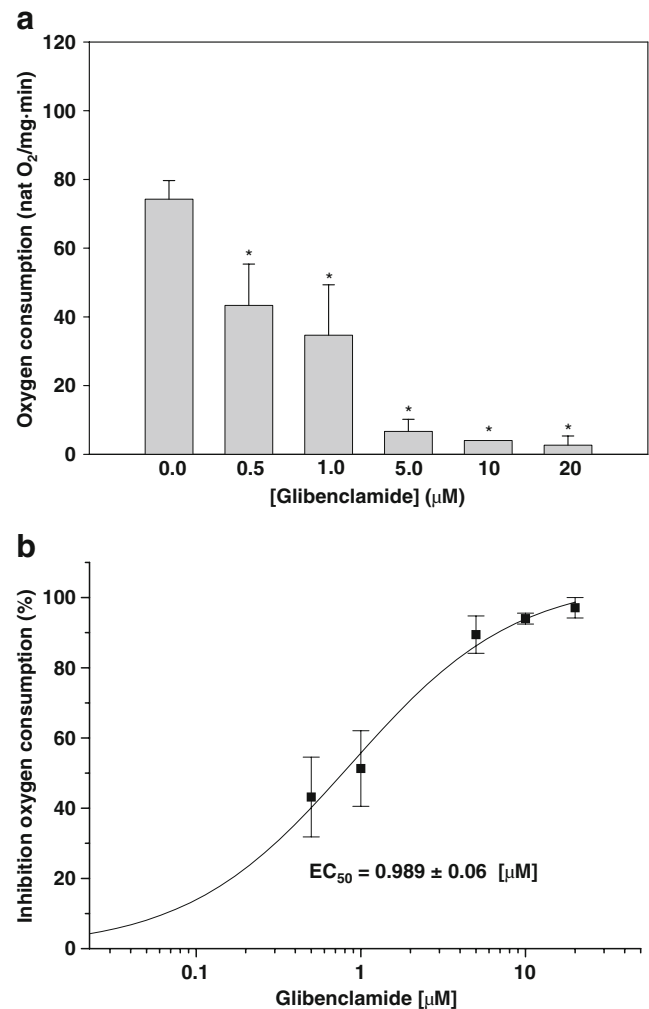
Fig. 1 **a** Effects of glibenclamide on oxygen consumption in state-3 respiration in isolated mitochondria. Oxygen consumption in nat of O_2/min.mg for Morelia City was 74.27±5.4. *Asterisks* represent significant values ($p < 0.05$) (n=4). **b** Dose-response curve fitted to Hill equation for glibenclamide. EC_{50} is 0.989±0.06 µM (n=4)

threshold was 0.5 μM, and maximum effect reached at 20 μM. Hill equation showed an EC_{50} of 0.989 ± 0.06 μM; I_{max} calculated value was 102.08 ± 2.48 μM, and Hill coefficient was 1.16 ± 0.14. However, when 5-HD was utilized at concentrations of 100 and 500 μM, it exerted no effect on mitochondrial respiration (data not shown).

Effect of pinacidil on mitochondrial oxygen consumption

To explore the action of pinacidil on mitochondrial oxygen consumption, succinate was used as substrate for mitochondrial respiration, but pinacidil had no effect on state-3 respiration in comparison to control (data not shown). However, when glutamate-malate was employed as substrate for complex I in all explored concentrations of pinacidil (1, 5, 10, and 50 μM), inhibited oxygen consumption (Fig. 2a). Fifty percent inhibition was

a

b

Fig. 2 **a** Effect of pinacidil on oxygen consumption in state-3 respiration in isolated mitochondria. Oxygen consumption was 107.2 ± 10 O$_2$/min.mg. *Asterisks* are values of $p<0.05$ ($n=4$). **b** Dose-response curve fitted to Hill equation for pinacidil. EC_{50} was 3.5 ± 1.14 μM ($n=4$)

obtained when pinacidil was 5 μM, while the higher inhibition (20%) of oxygen consumption was obtained with 50 μM pinacidil. However, pinacidil was negligibly effective on respiration inhibition compared with glibenclamide. Figure 2b depicts the concentration-response curve for the inhibition effect of pinacidil. The threshold concentration of pinacidil to inhibit oxygen consumption was ca. 1 μM, and the maximal effect was observed with 50 μM. Data adjusted to Hill equation showed an EC_{50} for pinacidil of 3.5 ± 1.14 μM. I_{max} value was 82.56 ± 8.30 μM, and Hill coefficient was 0.93 ± 0.19 μM. However, neither 5-HD nor glibenclamide exerted a significant effect on oxygen consumption when they were applied utilizing glutamate-malate as substrates for complex I (data not shown).

Effect of diazoxide on mitochondrial oxygen consumption

To assess whether diazoxide might alter mitochondrial function, we explored mitochondrial respiration in state 3 (Fig. 3). In Fig. 3a, the effect of diazoxide in the presence of succinate obtains maximal inhibition achieved with 50 μM diazoxide (approximately 30%), while with glutamate-malate, maximal inhibition reached with 100 μM was also 30% (Fig. 3b).

Discussion

In the present study, we explored oxygen consumption in isolated mitochondria derived from chicken pectoralis skeletal muscle in the presence of glibenclamide, pinacidil, 5-HD, and diazoxide (Jaburek et al. 1998) by using succinate and glutamate-malate as substrates for mitochondrial respiration. Simple addition of the drugs produced no changes in oxygen consumption when succinate was used as substrate, but when glutamate-malate was added, oxygen consumption was inhibited. In contrast, when 5-HD was utilized, it did not show a significant effect on mitochondrial oxygen consumption using both of the substrates (data not shown). In our experiments, glibenclamide diminished mitochondrial respiration only when mitochondria were absent from electron transport-chain uncouplers. Regarding these contrasting results and those reported for heart-isolated mitochondria, Jaburek et al. (1998) reported that inhibition of mitochondrial respiration derived from heart muscle using succinate as substrate in the presence of glibenclamide is due to a non-specific effect in uncoupled mitochondria, because glibenclamide is not a selective blocker for the mitochondrial K_{ATP} channel (Beavis et al. 1993).

Although glibenclamide has been explored in K_{ATP} channels, its action in blocking other types of K^+ channels,

a

b
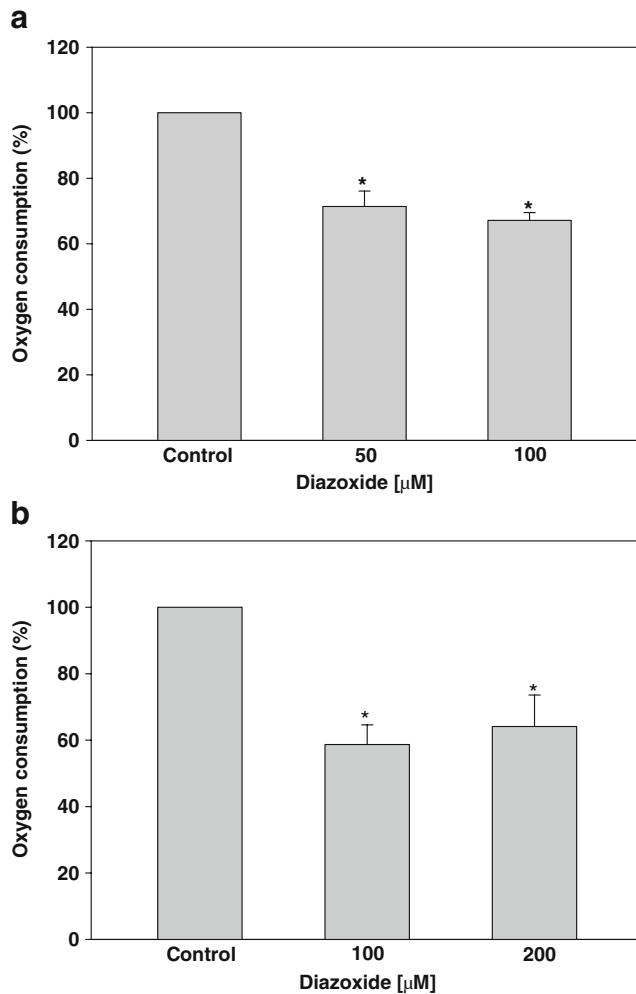
Fig. 3 Effect of diazoxide on oxygen consumption having in **a**, succinate as substrate, and in **b**, glutamate-malate. Results are shown on a normalized scale to 100%. *Asterisks* represent significant values ($p \leq 0.05$) ($n=4$)

as well as currents carried by TEA$^+$, could explain the non-specificity of its action (Jaburek et al. 1998). In addition, the high concentrations employed in several experiments or lower affinity to respiratory chain enzymes could explain the scarce specificity (Debeer et al. 1974; Somogyi et al. 1995a, b). In our experiments, the effect of glibenclamide could be a reflex of diminution in K$^+$ fluxes, which might not be relative to K$_{ATP}$ channels. Although we are unable to discard this, part of the effect of this drug could be due to blockade of the mitochondrial K$_{ATP}$ channels; additional research is needed to confirm this, for example, the use of another technical approach to explore the activity of single K$_{ATP}$ channels (via patch clamp) or direct measurements of potassium permeability (Hamill et al. 1981) in the presence of glibenclamide. Binding sites in the sulphonylurea-receptor protein phase from the K$_{ATP}$ channel could have effects that are dependent on the functional state of the channel (Schwanstecher et al. 1998).

Regarding the effects found by Jaburek et al. (1998), we have not observed any effect when 5-HD was utilized, despite our use of a—range of concentrations similar to those used by Mironova et al. (2004) to explore the action of this drug on mito-K$_{ATP}$. Jaburek et al. (1998) argue that binding of the drug would modify the channel's conformation, which would occur in vivo, but not in vitro, when succinate is the substrate employed. However, our results found with 5-HD are in agreement with those described for heart mito-K$_{ATP}$ (Garlid et al. 1997; Grover 1997). Hanley et al. (2005) showed that 5-HD metabolizes to 5-hydroxydecanoyl-CoA, which acts as substrate for the first step of β-oxidation, an action that could explain why no effect was described when this drug was used in cardiac preparations.

On the other hand, in previous studies, the use of pinacidil and succinate as substrate exerted no effects on oxygen consumption (Hanley et al. 2002) when NADH (800 μM) was used as substrate for submitochondrial particles from guinea pig heart, and pinacidil (23–230 μM) exhibited a concentration-dependent diminution in oxygen consumption with an EC$_{50}$ of 90 μM. These results suggested that pinacidil inhibits complex I (NADH-ubiquinone oxidoreductase). In our study, we used glutamate-malate as substrate for complex I, and pinacidil inhibited oxygen consumption in a concentration-dependent manner (EC$_{50}$=3.5±1.14 μM). Our concentration range utilized for pinacidil was smaller than those previously reported by Holmuhamedov et al. (1998) and Kowaltowski et al. (2001), although, a pharmacological effect could be considered to occur with a dose <50 μM (Holmuhamedov et al. 1998, 1999), which is higher than that we used, in which an inhibition of oxygen consumption was obtained (1 μM) (Fig. 2a). However, part of this effect might be due to the hydrophobic nature of pinacidil (Kowaltowski et al. 2001).

Additionally, although drugs could activate some complexes of the respiratory chain, they could also affect the transmembrane potential, and possibly Ca^{2+} uptake (Holmuhamedov et al. 1998, 1999), because the mito-K$_{ATP}$ channel regulates mitochondrial volume. In the absence of ATP, mito-K$_{ATP}$ channels are open and mitochondria uptake K$^+$ from the outside, which passes through the channel accompanied by water; an effect that is inhibited by ATP (Holmuhamedov et al. 1999).

Modulation of mito-K$_{ATP}$ by ATP is suggested to be a process that could regulate complete mitochondrial function and, consequently, energy production, and mitochondria convert from energy-producing organelles to an energy-consuming organelles, influencing cell response according to metabolic demand. In the cardiac myocyte, it has been shown that this mechanism is activated by dissipating mitochondrial inner-transmembrane potential, such as takes

place during mitochondrial permeability transitions (Duchen 1999), ischemia (Vuorinen et al. 1995), or during spontaneous redox oscillations (Romashko et al. 1998).

With respect to the effect of diazoxide on oxygen consumption, some K_{ATP} channel openers exert a direct effect on certain respiratory-chain proteins, such as the Succinate dehydrogenase enzyme (SDH) (Hanley et al. 2002), that is, SDH is inhibited with 100 μM diazoxide (Kowaltowski et al. 2001), a dose not much higher than that required to close mitoK_{ATP} channels (30 μM). Ardehali et al. (2005) showed that mito-K_{ATP} is part of the SDH macro-molecular complex, which modulates channel activity via a physical interaction, and not by playing a concrete role in the respiratory chain. Thus, our results are in agreement, because inhibition of the oxygen consumption succeeds in the presence of diazoxide (50 μM), a dose that falls within the limit of effects observed on respiratory chain compounds, whose existence may represent a direct correlation demon-strating that these effects are exerted on SHD.

We agree that neither pinacidil, glibenclamide, nor 5-HD are specific modulators of potassium conductance; their effects in mitochondria show dependence on the substrate used, but more research is required to establish via proteomic or via other studies the identity of K_{ATP}-channel proteins in mitochondria (Suzuki et al. 1997; Zhou et al. 2005). However, to our knowledge, this is the first report that K_{ATP}-channel agonists and antagonists affect oxygen consumption. Thus, our data suggest that, at least in part, these agents exert other effects on skeletal-muscle mito-chondria that are unrelated to their effects on the mito-K_{ATP} channel, which needs to be investigated.

References

Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP 4th, Boyd AE 3rd, González G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA (1995) Science 268:423–426

Allen DG (2009) J Appl Physiol 106:358–359

Allen DF, Lamb GD, Westerblad H (2008) Physiol Rev 88:287–332

Ardehali H, O'Rourke B, Marbán E (2005) Circ Res 97:740–742

Barre H, Berne G, Brebion P, Cohen-Adad F, Rouanet JL (1989) Am J Physiol 256:1192–1199

Beavis AD, Lu Y, Garlid KD (1993) J Biol Chem 268:997–1004

Brooks GA, Brown MA, Butz CE, Sicurello JP, Dubouchaud H (1999) J Appl Physiol 87:1713–1718

Calderón-Cortés E, Cortés-Rojo C, Clemente-Guerrero M, Manzo-Ávalos S, Villalobos-Molina R, Boldogh I, Saavedra-Molina A (2008) Mitochondrion 8:262–272

Dahlem YA, Wolf G, Siemen D, Horn T (2006) Cell Calcium 39:387–400

Das M, Parker JE, Halestrap AP (2003) J Physiol 547:893–902

Debeer L, Mannaerts G, De Schepper PJ (1974) Biochem Pharmacol 23:251–258

Debska G, Kicinska A, Skalska J, Szewczyk A, May R, Elger C, Kunz W (2002) Biochem Biophys Acta 1556:97–105

Duchen MR (1999) J Physiol 516:1–17

Eu JP, Hare JM, Hess DT, Skaf M, Sun J, Cardenas-Navina I, Sun QA, Dewhirst M, Meissner G, Stamler JS (2003) Proc Natl Acad Sci USA 100:15229–15234

Garlid KD, Paucek P (2001) IUBMB Life 52:153–158

Garlid KD, Paucek P, Yarov-Yarovoy HN, Murray RB, Darbenzio AJ, D'Alonzo NJ, Lodge NJ, Smith MA, Grober GJ (1997) Circ Res 81:1072–1082

Gornall AG, Bardawill CJ, David MM (1949) J Biol Chem 177:751–766

Green DR, Reed JC (1998) Science 281:1309–1312

Grover GJ (1997) Can J Physiol Pharm 75:309–335

Grover GJ, Garlid KD (2000) J Mol Cell Cardiol 32:677–695

Halestrap AP (1989) Biochem Biophys Acta 973:355–382

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Pflugers Arch 391:85–100

Hanley PJ, Mickel M, Loffler M, Brandt U, Daut J (2002) J Physiol 542:735–741

Hanley PJ, Dröse S, Brant U, Lareau RH, Banerjee AL, Srivastava DK, Banaszak LJ, Bsrychi JJ, Van Veldhoven PP, Daut J (2005) J Physiol 562:307–318

Holmuhamedov EL, Jovanovic S, Dzeja PP, Jovanovic A, Terzic A (1998) Am J Physiol 275:1567–1576

Holmuhamedov EL, Wang L, Terzic A (1999) J Physiol 519:347–360

Hoppeler H, Vogt M, Weibel ER, Flück M (2003) Exp Physiol 88:109–119

Huerta M, Stefani E (1981) J Physiol 318:181–189

Jaburek M, Yarov-Yarovoy V, Paucek P, Garlid KD (1998) J Biol Chem 273:13578–13582

Kowaltowski AJ, Seetharaman S, Paucek P, Garlid KD (2001) Am J Physiol 280:649–657

Mironova GD, Negada AE, Marinov BS, Paucek P, Costa ADT, Grigoriev SM, Skarga YY, Garrid KD (2004) J Biol Chem 279:32562–32568

Nicholls DG (2005) Cell Calcium 38:311–317

Nicholls DG, Ferguson SJ (2002) Bioenergetics 35. Academic, London, pp 89–154

O'Rourke B (2000) Circ Res 87:845–855

O'Rourke B, Ramza BM, Marbán E (1994) Science 265:962–966

Paucek P, Mironova G, Mahdi F, Beavis AD, Woldegiorgis G, Garlid KD (1992) J Biol Chem 267:26062–26069

Romashko DN, Marbán E, O'Rourke B (1998) Proc Natl Acad Sci USA 95:1618–1623

Sasaki N, Sato T, Marbán E, O'Rourke B (2001) Am J Physiol Heart Circ Physiol 280:H1882–H1888

Sato T, O'Rourke B, Marbán E (1998) Circ Res 83:110–114

Schwanstecher M, Sieverding C, Dörschner H, Gross I, Aguilar-Bryan L, Schwanstecher C, Bryan J (1998) EMBO J 17:5529–5535

Sims NR (1990) J Neurochem 55:698–707

Somogyi J, Ver A, Troja G, Vegh E, Banyasz T, Csermely P, Popovic S, Kovacs T (1995a) Acta Physiol Hung 83:313–321

Somogyi J, Ver A, Troja G, Vegh E, Buhler C, Hatfaludi F, Csermely P, Popovic S (1995b) Acta Physiol Hung 83:299–312

Suzuki M, Kotake K, Fujikura K, Inagaki N, Suzuki T, Gonoi T, Seino S, Takata K (1997) Biochem Biophys Res Commun 241:693–697

Talbot DA, Hanuise N, Rey B, Rouanet JL, Duchamp C, Brand MD (2003) Biochem Biophys Res Commun 312:983–988

Talbot DA, Duchamp C, Rey B, Hanuise N, Rouanet JL, Sibille B, Brand MD (2004) J Physiol 558:123–135

Thakar JH, Hassan MN (1988) Life Sci 43:143–149

Tricarico D, Barbieri M, Camerino DC (2000) Ann Neurol 48:304–312

Vander Heide RS, Hill ML, Reimer KA, Jennings RB (1996) J Mol Cell Cardiol 28:103–112

Vuorinen K, Ylitalo K, Peuhkurinen K, Raatikainen P, Ala-Rämi A, Hassinen IE (1995) Circulation 91:2810–2818

Zhou M, Tanaka O, Sekiguchi M, He H-J, Yasuoka Y, Itoh H, Kawahara K, Abe H (2005) J Histochem Cytochem 53:1491–1500