Pflügers Arch – Eur J Physiol (1999) 437:417–422

ORIGINAL ARTICLE

R.A. Venosa · A. Hoya

Effect of caffeine on K^+ efflux in frog skeletal muscle

Received: 13 March 1998 / Received after revision: 17 June 1998 / Accepted: 14 September 1998

Abstract The exposure of frog skeletal muscle to caffeine (3–4 mM) generates an increase of the K^+ ($^{42}K^+$) efflux rate coefficient ($k_{K,o}$) which exhibits the following characteristics. First it is promoted by the rise in cytosolic Ca^{2+} ($[Ca^{2+}]_i$), because the effect is mimicked by ionomycin (1.25 μM), a Ca^{2+} ionophore. Second, the inhibition of caffeine-induced Ca^{2+} release from the sarcoplasmic reticulum (SR) by 40 μM tetracaine significantly reduced the increase in $k_{K,o}$ ($\Delta k_{K,o}$). Third, charybdotoxin (23 nM), a blocker of the large-conductance Ca^{2+}-dependent K^+ channels (BK_{Ca} channels) reduced $\Delta k_{K,o}$ by 22%. Fourth, apamin (10 nM), a blocker of the small-conductance Ca^{2+}-dependent K^+ channels (SK_{Ca} channels), did not affect $\Delta k_{K,o}$. Fifth, tolbutamide (800 μM), an inhibitor of K_{ATP} channels, reduced $\Delta k_{K,o}$ by about 23%. Sixth, Ba^{2+}, a blocker of most K^+ channels, did not preclude the caffeine-induced $\Delta k_{K,o}$. Seventh, omitting Na^+ from the external medium reduced $\Delta k_{K,o}$ by about 40%. Eight, amiloride (5 mM) decreased $\Delta k_{K,o}$ by 65%. It is concluded that the caffeine-induced rise of $[Ca^{2+}]_i$ increases K^+ efflux, through the activation of: (1) two channels (BK_{Ca} and K_{ATP}) and (2) an external Na^+-dependent amiloride-sensitive process.

Key words Caffeine · Calcium · Efflux · Frog · Potassium · Skeletal muscle

Introduction

In skeletal muscle fibers the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is maintained at a submicromolar level by the Ca^{2+}-ATPases of the sarcolemma and the sarcoplasmic reticulum (SR) as well as by the sarcolemmal Na^+/Ca^{2+} exchanger. Increases of one to two orders of magnitude in $[Ca^{2+}]_i$ are easily produced by the release of small amounts of Ca^{2+} from the SR induced by caffeine. This will, in turn, generate a muscle fiber contracture as well as the activation of Ca^{2+}-ATPases and the Na^+/Ca^{2+} exchanger [9].

We found that, in addition to those effects, the caffeine-induced rise in $[Ca^{2+}]_i$, produces a large increase in K^+ efflux, to our knowledge not previously described, which can be reasonably explained in terms of the activation of two K^+ channels (BK_{Ca} and K_{ATP}) and a K^+ efflux which is dependent on external Na^+ and amiloride sensitive.

R.A. Venosa (✉) · A. Hoya
Centro de Investigaciones Cardiovasculares,
Facultad de Ciencias Médicas,
Universidad Nacional de La Plata, Calle 60 y 120,
1900 La Plata, Argentina
Fax: +54-21-255861

Materials and methods

Experiments were performed on isolated frog sartorius muscles from *Leptodactylus ocellatus*. Animals were chilled to full immobility in an ice-water mixture and subsequently double pithed. The isolated muscles were attached to thin purpose-made stainless-steel holders which kept them at a slack length while allowing free access of bathing media to the preparations. The normal Ringer solution had the following composition (mM): NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; Na_2HPO_4, 2.15; NaH_2PO_4, 0.85; pH=7.2. Tris-Cl buffer (5 mM) was used instead of phosphate buffer when $BaCl_2$ (5 mM) was added to the Ringer. In some experiments a Na^+-free medium of the following composition was used (mM): Tris-Cl, 119; KCl, 2.5; $CaCl_2$, 18; pH=7.2. Under these conditions increases in Ca^{2+} concentration were made by isosmotic replacements of Tris-Cl by $CaCl_2$.

Caffeine was purchased from Fluka, Switzerland and charybdotoxin (CTX) and ionomycin from Calbiochem, San Diego, Calif., USA. Tolbutamide was a gift from Dr. J.J. Gagliardino. Tris, tetracaine and amiloride were from Sigma. Ionomycin and amiloride were dissolved in dimethylsulfoxide (DMSO) before being added to the experimental solutions. No effect of the solvent per se on K^+ efflux could be detected.

K^+ efflux was measured using $^{42}K^+$ which was obtained from Comisión Nacional de Energía Atómica (Argentina). Muscles were first loaded with $^{42}K^+$ by bathing them in normal Ringer solution labelled with the isotope (≈740 kBq·ml^{-1} or 0.02 mCi·ml^{-1}) for 2.5–3.5 h. After the loading period, the preparations were prewashed for 60 min in unlabelled Ringer solution and the radioactivity released during this period was discarded. Subsequently, the muscles were washed in a series of rotating (60 turns per min) tubes containing 3 ml of unlabelled media at intervals of 5–15 min. At the end of the experiment each muscle was kept

in a tube containing 3 ml of distilled water. The radioactivity of the washout tubes and of those containing the muscles was determined using a gamma-counter (Tracor analytic 1191). The correction for radioactive decay (half-time: 12.4 h) and the rate of release of $^{42}K^+$ was calculated using a microcomputer and expressed in terms of efflux rate coefficient ($k_{K,o}$; in units of min^{-1}).

ATP and creatine phosphate (CP) were determined using the method of Lamprecht et al. [14].

Values from identical experiments are expressed as means ±1 SEM. Student's *t*-test was used to estimate the statistical significance of differences.

Results

An effective way of increasing $[Ca^{2+}]_i$ in frog muscle fibers is to promote the release of this cation from the SR by exposing them to an adequate concentration of caffeine. We used 3 and 4 mM caffeine. These concentrations of the drug produce moderate contractures and, as we found, increase $k_{K,o}$. Figure 1 shows a typical response of $k_{K,o}$ to 4 mM caffeine.

We are not aware of any direct effect of caffeine on K^+ permeability unrelated to the increase in $[Ca^{2+}]_i$. In order to test the contribution of the change in $[Ca^{2+}]_i$ to the observed effect on K^+ efflux, $[Ca^{2+}]_i$ was raised by promoting a net Ca^{2+} influx mediated by ionomycin, an ionophore for this cation. Figure 2A shows the response of $k_{K,o}$ to the addition of 1.25 μM ionomycin to Na^+-free Ringer ($[Ca^{2+}]_o$=1.8 mM). In these experiments, Na^+ was excluded (replaced by Tris) from the medium to favor the increase of $[Ca^{2+}]_i$ by preventing the activation of the Na^+/Ca^{2+} exchanger as $[Ca^{2+}]_i$ rose. However, it was found (see below) that the lack of external Na^+ has its own effect on $k_{K,o}$. As shown in Fig. 2B, in the presence of ionomycin, the magnitude of $\Delta k_{K,o}$ augmented monotonically with $[Ca^{2+}]_o$ (isosmotic replacement of Tris-Cl by $CaCl_2$). The exposure to 1.25 μM ionomycin produced a moderate contracture in the presence of 1.8 mM $[Ca^{2+}]_o$ similar to but slower than those observed in the presence of 4 mM caffeine (at higher Ca^{2+} concentrations they were somewhat more pronounced). Most likely, therefore, the stimulation of $k_{K,o}$ by caffeine is somehow the result of the increase in $[Ca^{2+}]_i$. As can be seen there was a small effect of the ionophore in the virtual absence of Ca^{2+} (0 Ca^{2+}; 1 mM EGTA). This may reflect either the presence of a low concentration of ionomycin in the cytosol, which would translocate Ca^{2+} from internal reservoirs (i.e., SR, mitochondria) into the cytosol, or a minor ability of this ionophore to transport K^+.

The faster time course of caffeine's effect on $k_{K,o}$ as compared with that of ionomycin could be due, in part, to the high permeability to caffeine and will be referred to later in the Discussion.

Quinine also releases Ca^{2+} from the SR [10]. In three identical experiments this drug (2 mM) produced, on average, an eightfold increase in $k_{K,o}$ (data not shown).

Local anesthetics are known to block caffeine-induced contractures [5]. The caffeine-induced Ca^{2+} release from the SR is significantly reduced by low concentrations of tetracaine [19]. We speculate that if the effect of caffeine on $k_{K,o}$ were mediated by the increase in $[Ca^{2+}]_i$, in the presence of tetracaine it should be reduced or abolished. A low concentration of tetracaine (40 μM) was used to avoid any significant direct effect of this anesthetic on K^+ permeability (P_K) while still significantly inhibiting the SR Ca^{2+}-release channel [25]. Figure 3 shows that tetracaine produced a marked reduction of the $\Delta k_{K,o}$ induced by caffeine. However, applying the same concentration of anaesthetic when the effect of caffeine

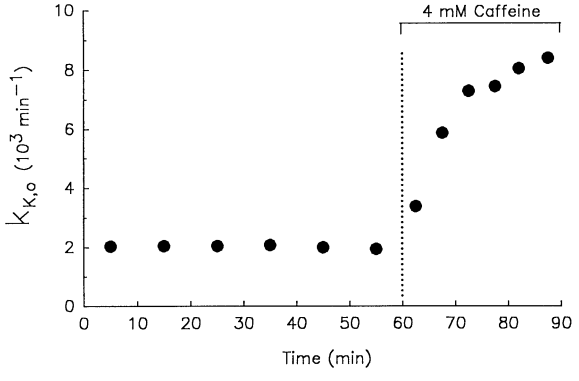

Fig. 1 Typical response of the K^+ efflux rate coefficient ($k_{K,o}$) from a sartorius muscle to exposure to 4 mM caffeine

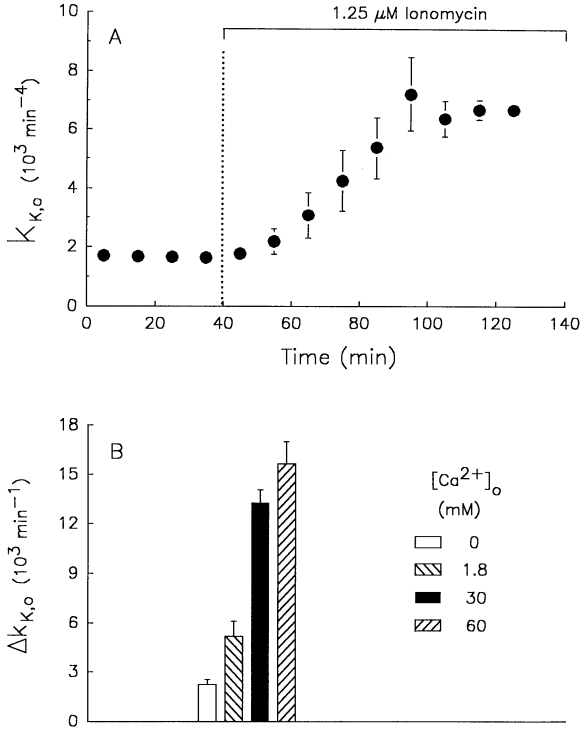

Fig. 2 **A** Effect of 1.25 μM ionomycin on $k_{K,o}$. Mean from four identical experiments. **B** Increase of $k_{K,o}$ ($\Delta k_{K,o}$) promoted by 1.25 μM ionomycin at 60 min from the beginning of the exposure to the ionophore in the presence of 0 (1 mM EGTA), 1.8, 30 and 60 mM $[Ca^{2+}]_o$. Na^+-free (Tris) Ringer. Each *bar* represents the mean from four identical experiments

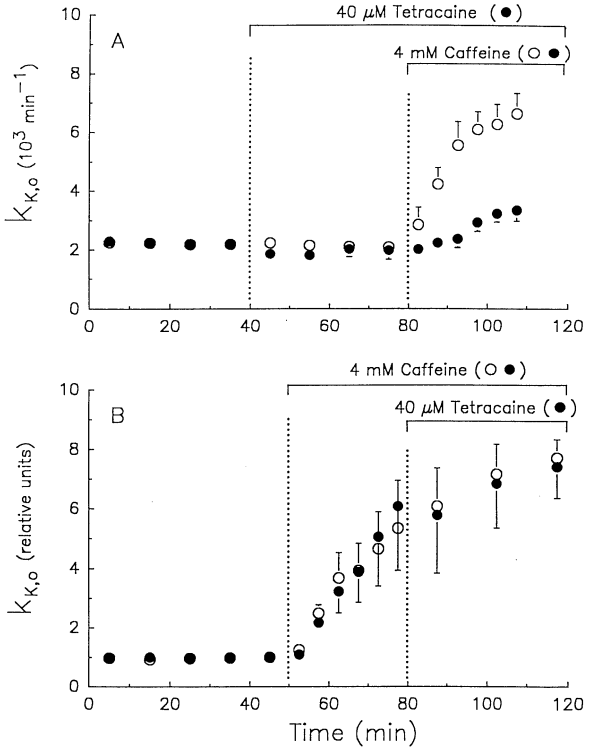

Fig. 3 **A** The effect of 40 μM tetracaine on the increase in $k_{K,o}$ induced by caffeine (●) as compared with controls (○). **B** Once the caffeine effect was established, exposure to tetracaine did not affect $k_{K,o}$ (●). The data indicate that tetracaine acts by blocking the caffeine-induced Ca^{2+} release and not the K^+ permeability. The data shown in each *panel* represent the means from four identical experiments carried out on paired control (○) and experimental (●) preparations

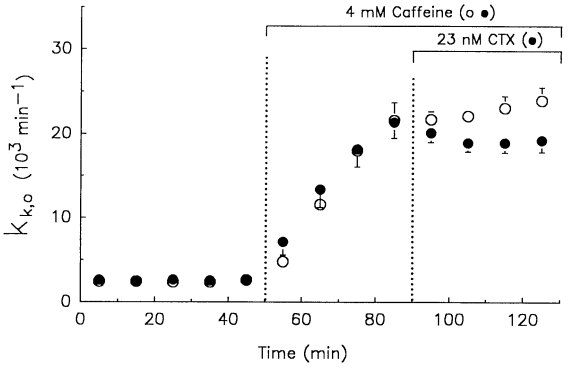

Fig. 4 Effect of charybdotoxin (*CTX*), an inhibitor of BK_{Ca}, on the increase of $k_{K,o}$ induced by 4 mM caffeine. Forty minutes after the application of 4 mM caffeine, the experimental muscles were exposed to 23 nM CTX. The 22% reduction of the caffeine effect produced by the toxin (●) was significant (P<0.05 for the last three paired values). Data from four identical experiments performed using paired preparations

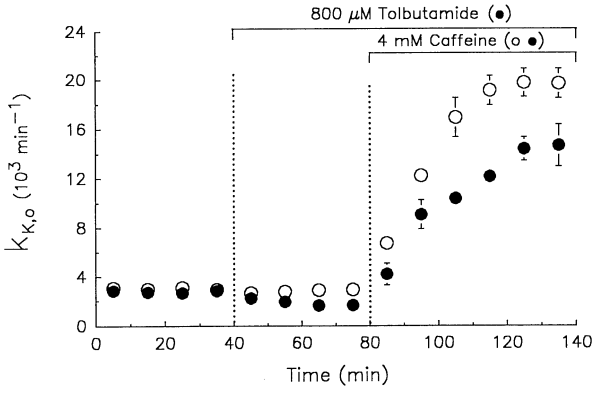

Fig. 5 Effect of tolbutamide, a blocker of the K_{ATP} channels, on $k_{K,o}$. The addition of 800 μM of the drug to the normal Ringer at the 40 min mark produced a 44% reduction in $k_{K,o}$ (P<0.05 for the last three paired values before caffeine). In the experimental muscles (●) the exposure to 4 mM caffeine produced a $\Delta k_{K,o}$ (with respect to the last value before caffeine) 23% lower than in control muscles (○) (P<0.03; 0.06 and <0.05 for the last three paired values; n=4)

on K^+ efflux was well developed failed to affect $\Delta k_{K,o}$, indicating that P_K was not altered.

Two types of Ca^{2+}-activated K^+ channels have been characterized in several preparations: one of small conductance – the SK_{Ca} channel, which is blocked by the honey-bee venom apamin – and the other of large conductance, the BK_{Ca} channel, selectively blocked by CTX. In an attempt to dissect the components of the $\Delta k_{K,o}$ promoted by caffeine we used both of these toxins. Apamin binds to several tissues including skeletal muscle with a K_D of 15–60 pM [20] and effectively blocks a Ca^{2+}-dependent component of the slow outward current on frog skeletal muscle [26]. Apamin (10 nM) did not affect $\Delta k_{K,o}$ (data not shown). The effect of 23 nM CTX, on the other hand, was rather modest. This concentration of CTX is about one order of magnitude greater than the apparent dissociation constant (1–3.5 nM) of the toxin from its receptor [7, 22, 27].

Figure 4 shows the effect of CTX. The reduction of Δk_{Ko} towards the end of the exposure to CTX was about 22%.

It has been shown [8] that in the presence of 5 mM caffeine frog muscles are rapidly depleted of high-energy phosphate compounds. We measured the ATP and CP contents in four sartorii which had been exposed to 4 mM caffeine for 15 min and also in their matched pair kept in normal Ringer (controls). ATP was reduced from an average of 3.90±0.40 μmol·g^{-1} in control muscles to 0.77±0.08 μmol·g^{-1} in caffeine-treated ones. Similarly, CP content fell from 8.90±0.72 μmol·g^{-1} to 0.93±0.08 μmol·g^{-1}. The fact that caffeine produces both a reduction of ATP content and a rise in $k_{K,o}$ suggests the involvement of ATP-dependent K^+ channels (K_{ATP} channels), first described by Noma [18], in the response of K^+ efflux to application of the drug. Under resting conditions, presumably, K_{ATP} channels of skeletal muscle, as in other cell types, are closed most of the time but their open probability increases as $[ATP]_i$ decreases [23]. The sulfonylurea derivative tolbutamide is a well-known inhibitor of these channels. We studied the effect of 800 μM tolbutamide. This is shown in Fig. 5. The drug reduced the resting $k_{K,o}$ by about 40% (P<0.05 for the last two paired values before caffeine), suggesting that a sizeable

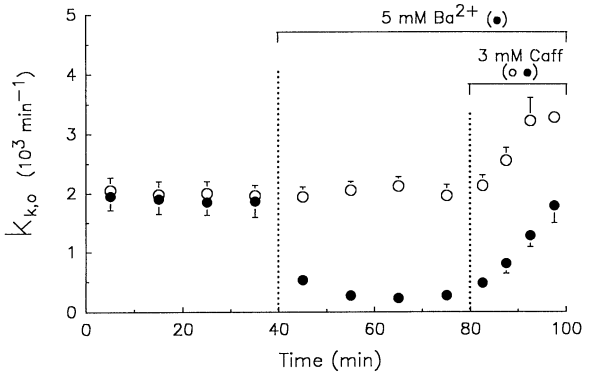

Fig. 6 Ba^{2+}, a blocker of most K^+ channels, does not suppress the increase in $k_{K,o}$ promoted by the rise of $[Ca^{2+}]_i$. The exposure of the experimental muscles (●) to 5 mM Ba^{2+} drastically reduced $k_{K,o}$. The subsequent application of 3 mM caffeine induced an increase of K^+ efflux, similar to that observed in control muscles (○); n=4

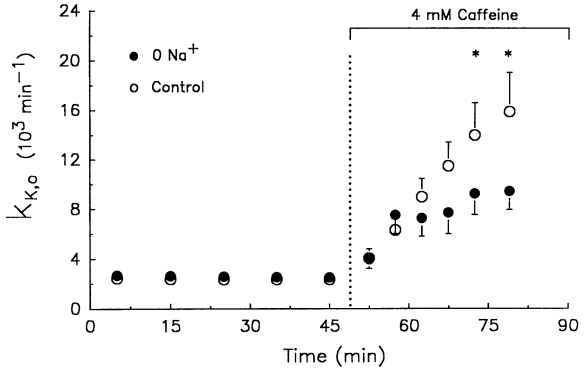

Fig. 7 Effect of Na^+-free (Tris) medium on the caffeine-induced $\Delta k_{K,o}$ in paired muscles. *P<0.05 (n=4)

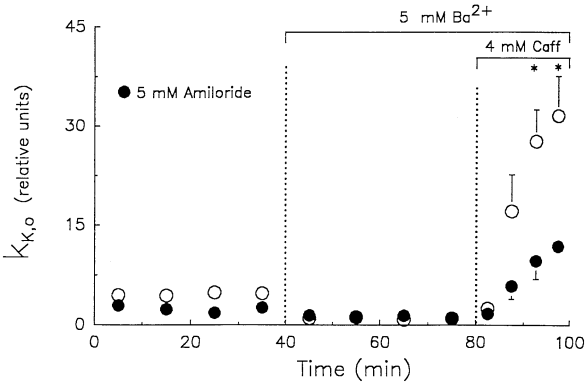

Fig. 8 Effect of 5 mM amiloride on the caffeine-induced $\Delta k_{K,o}$ in paired muscles in the presence of 5 mM Ba^{2+}. Means from four identical experiments (*P<0.02)

fraction of these channels are open in quiescent muscle fibers. On the other hand, the $\Delta k_{K,o}$ produced by 4 mM caffeine was reduced by tolbutamide by aproximately 23% (P<0.05; =0.06 and <0.05 for the last three paired values). In control experiments (not shown) the effect of 400 μM tolbutamide was similar in magnitude to that produced by 800 μM. The moderate inhibition of this drug is consistent with results of Castle and Haylett [1] in metabolically exhausted frog muscle.

External Ba^{2+} is a non-selective blocker of most K^+ channels [2]. As is shown in Fig. 6, Ba^{2+} (5 mM) dramatically reduced the resting $k_{K,o}$ which at 75 min amounted to 86±2%. The subsequent application of caffeine (3 mM) produced a substantial increase in $k_{K,o}$ in the absence as well as in the presence of Ba^{2+}. Thus, at the end of this run the inhibition by Ba^{2+} fell to 46±7%. At this point $k_{K,o}$ was not steady but still rising; therefore, the effect of Ba^{2+} should not be quantitatively compared with that before the addition of caffeine.

As mentioned before, the experiments of Fig. 2 were performed using Na^+-free media to block the forward activity of the Na^+/Ca^{2+} exchanger and thus to enhance the rise of $[Ca^{2+}]_i$ in the presence of ionomycin. Later on, however, control experiments revealed that a normal $[Na^+]_o$ did not reduce the $\Delta k_{K,o}$ induced by ionomycin. Furthermore, it was found that the caffeine-induced $\Delta k_{K,o}$ is diminished in Na^+-free medium. Figure 7 illustrates this point. It can be seen that in the absence of external Na^+ the response of $k_{K,o}$ after a 30-min exposure to caffeine was about 40% lower than in the presence of normal $[Na^+]_o$. This suggests that the exit of K^+ was somehow associated with the entry of Na^+.

It has been shown that the caffeine-induced Na^+/Ca^{2+} exchange in frog skeletal muscle is reduced by about 80% by 5 mM amiloride [9]. The Na^+ dependence of $\Delta k_{K,o}$ promoted by the methylxanthine suggests the possibility that Δk_K may also be affected by amiloride. Figure 8 shows that with most of the K^+ channels blocked by Ba^{2+}, amiloride reduced by about 65% the $\Delta k_{K,o}$ generated by caffeine. These results, which may suggest coupled Na^+/K^+ exchange, should be interpreted cautiously, because, although they have not been described in studies of skeletal muscle, epithelial cells have amiloride-sensitive and Ca^{2+}-activated non-selective channels which do not discriminate between Na^+ and K^+ and which could play a role here [6, 17]. In connection with this, it can be seen that amiloride alone (first 40 min in Fig. 8) reduced $k_{K,o}$, which represents a decrease in the resting K^+ permeability (P_K). Incidentally, this is an indication that in the presence of amiloride the sarcolemma did not deteriorate, otherwise an increase in $^{42}K^+$ leak would have been observed. Upon addition of Ba^{2+}, which blocks about 90% of P_K, $k_{K,o}$ both in control and amiloride-exposed muscles diminished and there was no longer any difference between them. Ba^{2+} blocks a host of K^+ channels very effectively, not only in excitable but also in epithelial cells [12]. Therefore, taken together, the reduction of the caffeine-induced $\Delta k_{K,o}$ by amiloride and by zero external Na^+, tentatively, may represent inhibition of either a transporter (K^+/Na^+ antiport?) or a non-selective (K^+/Na^+) Ba^{2+}-insensitive amiloride-sensitive ionic channel.

Discussion

We have studied the $\Delta k_{K,o}$ produced by caffeine. This drug inhibits the phosphodiesterase and consequently raises cAMP. Nonetheless, the effect described here does not seem to be caused by the increase in the intracellular level of the nucleotide but rather the rise of $[Ca^{2+}]_i$ subsequent to Ca^{2+} release from the SR. This is supported by the fact that the increase in $k_{K,o}$ also occurs when the rise in $[Ca^{2+}]_i$ is promoted by the translocation of extracellular Ca^{2+} mediated by ionomycin. The time course of the effect of caffeine was considerably faster than that of ionomycin even though the ionophore binds to the sarcolemma, while the binding sites for caffeine are in the SR. Two factors contribute to make the caffeine stimulation faster than the effect of ionomycin on $k_{K,o}$: (1) the high caffeine permeability (similar to P_{Cl}, [13]); (2) caffeine acts on a fast transport system like the SR Ca^{2+}-release channels. On the other hand, the incorporation of ionomycin into the membrane as well as the translocation of Ca^{2+} by the ionophore are two processes considerably slower than those involved in the caffeine effect.

That the caffeine-induced release of Ca^{2+} from the SR triggers a change in $k_{K,o}$ is further supported by the fact that, at a low concentration (40 μM), tetracaine, an inhibitor of caffeine-induced SR Ca^{2+} release, significantly reduced the effect, without affecting P_K. Two pieces of evidence indicate that the decrease of $\Delta k_{K,o}$ produced by tetracaine is caused by the reduction of the Ca^{2+} released by the SR and not by a decline of K^+ permeability. First, prior to the application of caffeine, tetracaine had no significant effect on $k_{K,o}$ (Fig. 3A). Second, application of the same concentration of tetracaine did not affect the $\Delta k_{K,o}$ induced by caffeine when the effect was well developed (Fig. 3B), meaning that this concentration of the anesthetic does not significantly affect P_K. In addition, it can be inferred from the data in Fig. 3A that 40 μM tetracaine does not completely abolish the caffeine-induced Ca^{2+} release. Thus, most likely, $[Ca^{2+}]_i$ remained high during the exposure to tetracaine in Fig. 3B, thereby keeping $k_{K,o}$ high.

Initially, the results suggested the involvement of Ca^{2+}-activated K^+ channels and consequently it was expected that CTX and/or apamin would substantially reduce $\Delta k_{K,o}$. The data, however, showed that apamin had no effect and that CTX modestly inhibited the caffeine-induced $\Delta k_{K,o}$. This agrees well with data of Spruce et al. [24]. They did not find BK_{Ca} channels in the surface membrane. The presence of a low density of this type of channel in the T tubule membrane, however, is not unlikely. In fact, Latorre et al. [15] demonstrated their presence in the T tubules of rabbit skeletal muscle.

The fall in the content of high-energy phosphate compounds produced by caffeine prompted us to investigate the possible participation of K_{ATP} channels. Tolbutamide (800 μM), a blocker of these channels, produced a relatively greater reduction of the resting $k_{K,o}$ (44%) than of $\Delta k_{K,o}$ produced by caffeine (23%). This suggests, at variance with data from other cell types, that K_{ATP} channels contribute substantially to the resting P_K, as if the resting open probability of these channels in skeletal muscle fibers of the presently studied species (*Leptodactylus ocellatus*) were higher than that in most other cell types. In frog muscle from *Rana temporaria,* Davies [4] found a K_D for the ATP block of K_{ATP} channels of 0.14 mM. Our data show that in the presence of caffeine $[ATP]_i$ fell to 0.77 μmol·g^{-1} or about 1.28 mM (assuming that the intracellular water represents about 60% of the muscle mass). Since under these conditions $\Delta k_{K,o}$ and resting $k_{K,o}$ were reduced by tolbutamide, we suspect that the K_D in our preparation may be considerably higher. It is noteworthy that, according to Spruce et al. [23], the density of these channels in the sarcolemma of frog muscle fibers is as high or higher than that of the delayed rectifier channels.

Ba^{2+} (5 mM), a non-specific blocker of most K^+ channels which reduced the resting $k_{K,o}$ by about 90% (see Fig. 6), did not preclude an increase of $k_{K,o}$ mediated by the caffeine-induced rise of $[Ca^{2+}]_i$.

An interesting and unforeseen result was that concerning the external Na^+ dependence of $\Delta k_{K,o}$. In principle this may suggest that the increase in $[Ca^{2+}]_i$ promotes a Na^+_o/K^+_i exchange. We have characterized the Na^+/Ca^{2+} exchange in frog skeletal muscle [9], where the activation of the exchanger by 4 mM caffeine produced an external-Na^+-dependent increase in Ca^{2+} efflux which was markedly reduced by 5 mM amiloride, a blocker of the exchanger in other tissues. (It has been shown that the fall of ATP produced by caffeine does not affect the activity of the skeletal muscle type of exchanger [16].) The present $\Delta k_{K,o}$, induced under similar experimental conditions, was also diminished by about 65% in the presence of the same concentration of amiloride. These data make us wonder whether K^+, in addition to Ca^{2+}, is exchanged for Na^+ in these fibers, as seems to be the case in retinal rods [3, 21] and platelets [11]. Nevertheless, the possibility of some sort of Na^+/K^+ exchange through the activation of Ca^{2+}_i-dependent non-selective cation channels of the type present in epithelial cells cannot be discarded, even though the presence of these channels in muscle, as yet, has not been reported.

In conclusion, we have found that the rise in cytosolic Ca^{2+} caused by caffeine-induced release of SR Ca^{2+} promotes an increase of K^+ efflux. The $\Delta k_{K,o}$ is not the result of an increase in the permeability of a single type of K^+ channel. The data indicate that about one-half of this increase can be ascribed to the rise in the P_K of K_{ATP} and BK_{Ca} channels. The rest of the caffeine-induced $\Delta k_{K,o}$ – which is Ba^+ insensitive, amiloride sensitive and, on account of its $[Na^+]_o$ dependence, somehow associated with a Na^+ influx – may represent a Na^+_o/K^+_i trade, whose nature deserves further investigation.

Acknowledgements This work was supported by CONICET (The National Research Council of Argentina) and the University of La Plata. We are indebted to Dr. Susana Mosca for the measurements of ATP and CP and to María C. Abel for her help in some of the experiments.

References

1. Castle NA, Haylett DG (1987) Effect of channel blockers on potassium efflux from metabolically exhausted frog skeletal muscle. J Physiol (Lond) 383:31–43
2. Castle NA, Haylett DG, Jenkinson DH (1989) Toxins in the characterization of potassium channels. Trends Neurosci 10: 59–65
3. Cerveto L, Lagnado L, Perry RJ, Robinson DW, McNaughton PA (1989) Extrusion of calcium from rod outer segments is driven by both sodium and potassium gradients. Nature 337: 740–743
4. Davies NW (1990) Modulation of ATP-sensitive K^+ channels in skeletal muscle and intracellular protons. Nature 343:375–377
5. Feinstein MB (1963) Inhibition of caffeine rigor and radiocalcium movements by local anesthetics in frog sartorius muscle. J Gen Physiol 47:151–172
6. Garty H (1994) Molecular properties of epithelial, amiloride blockable Na^+ channels. FASEB J 8:522–528
7. Giménez-Gallego G, Navia MA, Reuben JP, Katz GM, Kaczorowski GJ, García ML (1988) Purification, sequence and model structure of charybdotoxin, a potent inhibitor of calcium-activated potassium channels. Proc Natl Acad Sci USA 85:3329–3333
8. Hays ET, Connett RJ (1978) Effect of methylxanthines and elevated external potassium on high energy phosphate content in frog skeletal muscle. Biochem Pharmacol 27:2965–2968
9. Hoya A, Venosa RA (1995) Characteristics of the Na^+-Ca^{2+} exchange in frog skeletal muscle. J Physiol (Lond) 486:615–627
10. Isaacson A, Sandow A (1967) Quinine and caffeine effects on ^{45}Ca movements in frog sartorius muscle. J Gen Physiol 59: 2109–2120
11. Kimura M, Aviv A, Reeves JP (1992) K^+-dependent Na^+/Ca^{2+} exchange in human platelets. J Biol Chem 268:6874–6877
12. Kirk KL, Dawson DC (1983) Basolateral potassium channel in turtle colon: evidence for single-file ion flow. J Gen Physiol 82:297–313
13. Kotsias BA, Venosa RA, Horowicz P (1984) Caffeine contractures in denervated frog muscle in denervated frog muscle. Pflügers Arch 400:269–273
14. Lamprecht W, Stein P Heinz F, Weisser H (1974) Creatine phosphate. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic, vol. 4, New York, pp 1777–1781
15. Latorre R, Vergara C, Hidalgo C (1982) Reconstitution in lipid bilayers of a Ca^{2+}-dependent K^+ channel from transverse tubule membranes isolated from rabbit skeletal muscle. Proc Natl Acad Sci USA 70:805–809
16. Linck B, Qiu Z, He Z, Tong Q, Hilgemann DW, Philipson KD (1998) Functional comparison of the three isoforms of the Na^+/Ca^{2+} exchanger (NCX1, NCX2, NCX3). Am J Physiol 274: C415–C423
17. Nilius B (1997) Ion channels in nonexcitable cells. In: Sperelakis N (ed) Cell physiology, 2nd edn. Academic, New York, pp 436–455
18. Noma A (1983) ATP-regulated K^+ channels in cardiac muscle. Nature 305:147
19. Pizarro G, Csernoch L, Uribe I, Ríos E (1992) Differential effects of tetracaine on two kinetic components of calcium release in frog skeletal muscle fibres. J Physiol (Lond) 457: 525–538
20. Romey G, Hugues M, Schmid-Antomarchi H, Lazdunski M (1984) Apamin: a specific toxin to study a class of Ca^{2+}-dependent K^+ channels. J Physiol (Paris) 79:259–264
21. Schnetkamp PPM, Basu DK, Szerecsei RT (1989) Na-Ca exchange in the outer segments of bovine rod photoreceptors requires and transport potassium. Am J Physiol 257:C153–C157
22. Smith C, Phillips M, Miller C (1986) Purification of charybdotoxin, a specific inhibitor of the high conductance Ca^{2+}-activated K^+ channel. J Biol Chem 261:14607–14613
23. Spruce AE, Standen NB, Stanfield PR (1985) Voltage-dependent, ATP-sensitive potassium channels of skeletal muscle membrane. Nature 316:736–738
24. Spruce AE, Standen NB, Stanfield PR (1987) Studies of the unitary properties of adenosine-5'-triphosphate-regulated potassium channels of frog skeletal muscle. J Physiol (Lond) 382: 213–236
25. Strichartz GR, Ritchie JM (1987) The action of local anesthetics on ion channels of excitable tissues. In: Strichartz GR (ed) Handbook of experimental pharmacology, vol 81. Springer, Berlin Heidelberg New York, pp 21–53
26. Traoré F, Cognard C, Potreau D, Raymond G (1986) The apamin-sensitive potassium current in frog skeletal muscle: its dependence on the extracellular calcium and sensitivity to channel blockers. Pflügers Arch 407:199–203
27. Valdivia HH, Smith JS, Martin BM, Coronado R, Possani LD (1988) Charybdotoxin and noxiustoxin two homologous peptide inhibitors of the $K^+(Ca^{2+})$ channel. FEBS Lett 226: 280–284