Adrenergic Modulation of the K^+ Contractures in Tonic Skeletal Muscle Fibers of the Frog

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Abstract The effects of adrenaline and isoprenaline on K^+ contractures of curarized tonic skeletal fibers were investigated. The K^+ contractures of tonic fibers have a peak tension followed by a sustained tension. The peak tension and total tension (the tension-time integral—area— of K^+ contractures) were increased by adrenaline and isoprenaline. The resting potential of tonic skeletal fibers were unaffected by adrenaline. The calcium channel blocker (cadmium and nifedipine) greatly blocked the effects of adrenaline on the peak and total tension of K^+ contractures. On the other hand, the peak and sustained tensions of K^+ contractures were greatly reduced in Ca^{2+} -free solution, but, the peak tension recovered when the fibers were pre-incubated in adrenaline. It is proposed that adrenergic modulation of tension in tonic skeletal muscle fibers could be related with the modulation of Ca^{2+} channels and/or Ca^{2+} release from the sarcoplasmic reticulum.

Key words: tonic skeletal fibers, adrenergic modulation, K^+ contractures.

Frog skeletal muscles possess two main different types of muscle fibers: twitch and slow or tonic. Twitch fibers generate a transient contracture in high K^+ . In contrast, tonic fibers give a maintained K^+ contracture (TASAKI and MIZUTANI, 1944; KUFFLER and VAUGHAN WILLIAMS, 1953a, b). The K^+ contracture in the tonic fibers has an initial transient phase (peak tension) followed by sustained tension (HAYATSU *et al.*, 1981; HUERTA *et al.*, 1986). The initial phase is mediated by the translocation of intracellular Ca^{2+} from sarcoplasmic reticulum (SR) and the sustained phase depends on the presence of calcium in the external solutions (HUERTA *et al.*, 1986). In twitch fibers, the adrenaline potentiates twitch (OOTA and NAGAI, 1977; ARREOLA *et al.*, 1987) and tetanus also (GONZALEZ-SERRATOS

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et al., 1981). This potentiation of tension is due to the adrenergic modulation of calcium channels (ARREOLA *et al.*, 1987) or the SR Ca^{2+} pump via cyclic adenosine monophosphate (cAMP) (GONZALEZ-SERRATOS *et al.*, 1981). On the other hand, diverse studies have shown the presence of an inward Ca^{2+} current in tonic muscle fibers (HUERTA and STEFANI, 1983, 1986; ZACHAROVA *et al.*, 1985). In the present experiments, we have further analyzed the effects of adrenaline and isoprenaline on K^{+} contractures in tonic skeletal muscle fibers. A preliminary report has already been published (HUERTA *et al.*, 1988).

METHODS

Mechanical recording. Experiments were done on *Rana pipiens* kept at room temperature. Isometric tension measurements were performed on small bundles of tonic fibers (diameter $< 500 \mu\text{m}$) dissected from the cruralis muscle (GILLY, 1975). To record tension, the distal end of the bundle was fixed to the bottom of the experimental chamber, while the proximal end was attached to the lever of an isometric mechanoelectrical transducer (Cambridge Technology, Inc. 400). The transducer was wired to a rectilinear pen recorder (Gould 220). The fibers were stretched to 1.30 times slack length. The exchange of solution was performed by the procedure described by HUERTA *et al.* (1986). It was possible to record several K^{+} contractures with similar amplitude and time course in the same bundle. The bundles were allowed to rest for 30 min between contractures. The area under tension-time curve was measure using a computer (AST premium 386SX/16) equipped with digitizing tablet (Houston Instrument Hipad Plus) and Sigma Scan program. The numbers at the right of each record represent the integral of the area under tension-time curve. The values of the area were normalized to the initial control K^{+} contracture in each bundle.

Electrical recording. Conventional intracellular techniques were used to record from and inject current into cells using micropipettes. Recording pipettes were filled with 3 M KCl. Current-injecting pipettes were filled with 2 M potassium citrate. Electrical experiments were performed on the whole pyriformis muscle (STEFANI and STEINBACH, 1969; GILLY and HUI, 1980). Tonic muscle fibers were impaled with micropipettes (20–30 M Ω). The values of the resting potential reported corresponded to measurements taken immediately after the insertion of the micropipette, since the fibers were identified according to their passive electrical properties (STEFANI and STEINBACH, 1969).

Solutions. A composition of normal solution (mM): NaCl, 117.5; KCl, 2.5; CaCl_2 , 1.8, was used. Solutions with high K^{+} were obtained by equimolar replacement of NaCl with KCl. To reduce possible changes on the surface potential when Ca^{2+} was omitted from the solution, 1.8 mM CaCl_2 was replaced by 0.5 mM NiCl_2 (HILLE *et al.*, 1975). The muscle fibers were exposed to Ca^{2+} -free solution 5 min prior to 40 mM K^{+} . Nifedipine (Sigma Chemical Co.) was added from concentrated ethanol solutions (10 mM). The nifedipine solution was prepared

under a red light and the experiments were performed in the dark. Cadmium was taken from stock solution and added to the experimental solution in volumes needed to obtain the concentration desired. In all experiments, the muscles were curarized with $50\text{ }\mu\text{M}$ *d*-tubocurarine (Sigma Chemical Co.) to avoid a possible Ca^{2+} entry via acetylcholine channels, since they can be activated by a K^{+} -induced acetylcholine release during the contracture and prevent the action of adrenaline or isoprenaline on nerve terminals (JENKINSON and NICHOLLS, 1961; HUERTA *et al.*, 1986). When the bundles were not curarized, the potentiation effects of adrenaline were even greater. Adrenaline from Pisa Lab. and isoprenaline from Sigma Chemical Co. were used. The tonic bundles were pre-incubated with these drugs 10–20 min prior to the experiments. All solutions were buffered to pH 7.4 with 2 mM of imidazole chloride. Experiments were performed at room temperature (20–22°C). The values are given as mean $\pm$ standard error of the mean (S.E.). The number of observations is found in parentheses.

RESULTS

Effects of adrenaline on K^{+} contractions

Figure 1 is a representative result of effects of adrenaline on K^{+} contractions in curarized tonic muscle fibers. Record A shows a K^{+} contracture in normal solution (control contracture). The tension had an initial component (peak tension) which decayed to a relatively sustained level. After returning to normal solution, tension rapidly diminished. After 20-min exposure of the tonic bundle to adrenaline ($20\text{ }\mu\text{M}$), an increase of peak tension was observed ($43 \pm 16\%$, $n=4$). Figure 1B shows an increase of the peak tension by 58% with respect to record A. This potentiating effect of adrenaline on peak tension is associated with an increase of the sustained tension. If total tension is defined as the area under the K^{+} contracture profile, then adrenaline potentiates the tension by $40 \pm 15\%$ ($n=4$) with respect to the control. In the case of Fig. 1B, the increase of total tension was 46% in respect to the control. When the solution containing adrenaline was washed out, the contracture tension recovered to the same amplitude as the control (Fig. 1C). The potentiating effect of $1\text{ }\mu\text{M}$ adrenaline was less pronounced than with $20\text{ }\mu\text{M}$ (see Fig. 2A–C). It was important to study whether effects of the adrenaline were associated with membrane depolarizations, therefore, explaining this positive inotropic effect. In agreement with the data on twitch fibers (OOTA and NAGAI, 1977; GONZALEZ-SERRATOS *et al.*, 1981), we found that the resting potential of tonic fibers was not modified by adrenaline. The resting potential of tonic fibers in the normal solution was $-82 \pm 8\text{ mV}$ ($n=8$); with $1\text{ }\mu\text{M}$ adrenaline plus $50\text{ }\mu\text{M}$ *d*-tubocurarine added to normal solution it was $-85 \pm 7\text{ mV}$ ($n=14$); and with $20\text{ }\mu\text{M}$ adrenaline plus $50\text{ }\mu\text{M}$ *d*-tubocurarine it was $-82 \pm 4\text{ mV}$ ($n=13$).

Effect of isoprenaline on K^{+} contractions

It is known that isoprenaline acts predominantly on the β -adrenergic receptor.

Fig. 1. Effects of adrenaline (ADR) on K^+ contractures. A) Control contracture. B) Contracture elicited in the presence of $20 \mu M$ of adrenaline. C) Contracture after washing out the adrenaline. The numbers, associated to each record, represent the percentage of total tension (see METHODS) as compared to control.

In order to determine if the effect of adrenaline is mediated by β -adrenergic receptors, we studied the effect of isoprenaline on tonic muscle tension. Figure 2 shows a result of the effects of isoprenaline on K^+ contractures. When isoprenaline ($1 \mu M$) was added to the bathing solution, the peak tension and the total tension were increased up to 14 and 8% respectively as compared to control (Fig. 2E vs. D). On average, peak tension was $13 \pm 2\%$ and total tension $7 \pm 2\%$ ($n=6$). Adrenaline ($1 \mu M$) had an effect of increasing the peak tension and total tension by 19 and 35% respectively (Fig. 2B vs. A). On average, peak tension was $16 \pm 6\%$ and total tension was $32 \pm 10\%$ ($n=3$). This effect disappeared after washing the bundle in normal solution (Fig. 2C and F), although a little potentiation remains in

Fig. 2. Effects of adrenaline (ADR) and isoprenaline (ISO) on K^+ contractures. Records A and C show the control contractures for B. Record B demonstrates the potentiating effects of $1 \mu M$ of adrenaline. Records D and F show control contractures for E. Record E demonstrates the potentiating effects of $1 \mu M$ of isoprenaline. A bundle was used for records A-C and other bundle for records D-F.

total tension (5%).

Effect of Cd^{2+} on K^+ contractures in adrenaline-containing solution

The possible adrenergic modulation of Ca^{2+} inflow through calcium channels during K^+ contractures in tonic muscle fibers was tested by studying the effects of

Fig. 3. Effects of adrenaline (ADR) on K⁺ contractures in the presence of cadmium. A and C represent the initial and final control contractures. B shows the tension reduction effects of pre-incubation on a bundle in a solution containing both adrenaline (20 μM) and Cd²⁺ (2 mM).

blockers of Ca²⁺ channels such as cadmium and nifedipine (ALMERS and McCLESKEY, 1984; HUERTA and STEFANI, 1986). Figure 3 shows the effects of 2 mM of Cd²⁺ on K⁺ contractures in adrenaline-containing solution. Figure 3A and C are the control contractures; in record B, the bundle was incubated in normal solution plus adrenaline (20 μM) and Cd²⁺ (2 mM) before contracture. It is clear that the peak tension and total tension were reduced (67 and 60% respectively) in the presence of adrenaline and Cd²⁺. This effect was reversible (Fig. 3C) after washing out the adrenaline and Cd²⁺. Similar results were obtained in three bundles when the control was run in normal solution plus 2 mM Cd²⁺. The same sequence of results was obtained when the bundles were pre-incubated with nifedipine (5 μM). However, in experiments with nifedipine, its reduction was always only partially reversible to control tension.

Fig. 4. Effects of adrenaline (ADR) on K^+ contracture in Ca^{2+} -free solution. Record A is the control contracture. B shows the effects of Ca^{2+} -free solution on contracture. C corresponds to a contracture elicited in the presence of adrenaline ($20 \mu M$) in a Ca^{2+} -free solution. D is the contracture after washing out the adrenaline with normal solution.

Effect of adrenaline on K^+ contractures in Ca^{2+} -free solution

In skeletal muscle the increase of tension induced by adrenaline could be due to adrenaline acting on the SR. We investigated this possibility by studying the effect of adrenaline on tension development in Ca^{2+} -free solution. Figure 4A shows a 40 mM K^+ contracture in solution with Ca^{2+} 1.8 mM (control contracture). As previously reported by HUERTA *et al.* (1986), Fig. 4B illustrates the external Ca^{2+} dependence of the K^+ contracture when the tonic fibers are pre-soaked in a Ca^{2+} -free solution (see METHODS). It is clear that the peak tension is drastically reduced to 16% and total tension is also reduced to 5% of control contracture. However, under the same conditions when the bundles were pre-incubated in adrenaline ($20 \mu M$), the peak and total tension were recovered to 98 and 40% of control contracture, respectively (see Fig. 4C vs. A). On average, peak tension was $80 \pm 20\%$ and total tension, $40 \pm 10\%$ ($n=4$). The effects of Ca^{2+} -free solution (Fig. 4D) and also adrenaline were reversible.

DISCUSSION

The present results show that adrenaline and isoprenaline cause an increase of peak tension and total tension of K^+ contractures in tonic skeletal muscle fibers. These suggest the presence of β -adrenergic receptors in the membranes of these fibers. Adrenaline unaffected the resting potential in this fiber, therefore, the positive inotropic effect of adrenaline is not explained by a change in the resting potential.

The peak tension of K^+ contracture is mainly mediated by Ca^{2+} release from SR and most of the sustained tension depends on external Ca^{2+} (see introduction). External Ca^{2+} may act via the Ca^{2+} current described by HUERTA and STEFANI (1983) or by specific interaction with Ca^{2+} binding sites (BRUM *et al.*, 1988; GAMBOA-ALDECO *et al.*, 1988; TANABE *et al.*, 1990).

Along this line, the present results can be explained by adrenergic modulation of the Ca^{2+} current and/or by adrenergic modulation of Ca^{2+} release from SR. In the former, in our laboratory, preliminary results indicate that adrenaline ($1 \mu M$) increases the Ca^{2+} current in tonic muscle fibers by approximately 40% (MUÑOZ, 1990). Furthermore, the effect of $2 mM$ Cd^{2+} on K^+ contractures suggests that the potentiating effect of adrenaline on total tension could be, in part, due to an enhancement of the Ca^{2+} current during contracture; however, we could not discard additional effect of Cd^{2+} on membrane Ca^{2+} binding sites. There is a resemblance to previous reports about twitch tension potentiation in the twitch fibers and cardiac muscles produced by adrenaline associated with an increase of a slow inward calcium current, which is probably due to ion channel phosphorylation by cAMP (REUTER, 1983; ARREOLA *et al.*, 1987).

In the latter case, the effect of adrenaline on K^+ contractures in Ca^{2+} -free solution suggests that there is an adrenergic modulation of Ca^{2+} release from SR (see Fig. 4). These results agree with previous reports about the effect of adrenaline on the Ca^{2+} pump of the SR (GONZALEZ-SERRATOS *et al.*, 1981). Further study is necessary to understand the effects of adrenaline on modulation of Ca^{2+} release from SR. Alternatively, it is possible that adrenaline could be acting on the sarcolemmal site which regulates excitation-contraction coupling.

It is generally accepted that the β -adrenergic effect is mediated by the activation of adenylate cyclase, which causes an increase in the level of intracellular cAMP (RASMUSSEN and TENENHOUSE, 1968; BOWMAN and NOTT, 1969; OOTA and NAGAI, 1977; REUTER, 1987). However, in the tonic skeletal muscle fibers little is known about the effect of adrenaline on the ATP-yielding system. Further investigations on this problem are in progress.

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