Mechanisms of Slow Contracture Induced by Potassium and Caffeine in Skeletal Muscle of the Dog

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Accepted September 16, 1983

Abstract—Effects of diltiazem (3 μg-0.3 mg), verapamil (3 μg-0.3 mg), tetracaine (30 μg-3 mg), MnCl₂ (0.1 mg-10 mg) and CaCl₂ (0.1 mg-10 mg) on the skeletal muscle contracture induced by KCl and caffeine infusions were studied in the isolated, blood-perfused canine diaphragm preparation. All drugs were injected intra-arterially. Continuous intra-arterial infusion of KCl (50-100 mg/min) produced a biphasic contracture, which was characterized by a fast phasic contracture and a following tonic contracture. All drugs tested in this study except for CaCl₂ produced a dose-dependent relaxation in the state of tonic contracture induced by KCl infusion. Dose-response curves for the relaxation of muscle to these drugs were all in parallel. Diltiazem and verapamil were equipotent and were about 10 and 30 times more potent than tetracaine and MnCl₂ on a weight basis. Conversely, CaCl₂ produced a contractile response in a dose-dependent manner. On the other hand, diltiazem, verapamil and CaCl₂ had almost no effect on contracture induced by continuous intra-arterial infusion of caffeine (20 mg/min). MnCl₂ produced a slight relaxation in the caffeine-contracture with much higher doses than those against potassium-contracture. However, tetracaine produced a dose-dependent relaxation of caffeine-contracture. Thus, the results suggest that the entry of external calcium plays an essential role in the potassium-induced tonic contracture and that the potassium- and caffeine-contracts were maintained by different mechanisms.

Potassium-contracture of the skeletal muscle has been studied using excised muscle preparations of the frog (1-3), mouse (4, 5) and chicken (6, 7); and some role of external calcium in maintaining the tension during the contracture has been suggested. Recently, we have developed a method to investigate the excised diaphragm of the dog supported by blood perfusion (8). Continuous intra-arterial infusion of potassium chloride to this preparation produced a contracture strongly resembling that reported with mouse muscle (5). Subsequently we have studied the potassium-contracture of the dog diaphragm preparation pharmacologically and reported that a calcium antagonist, verapamil, effectively produced a relaxation of the slow contracture, implicating the active role of the slow channels in this type of contracture (9). Although studies on excitation-contraction coupling have been done exclusively with single fiber preparations or excised muscle as thin as possible in order to enable gas exchange and nutrition for the muscle cells, the blood-perfused myocardial preparation has been proven to be superior over the preparations immersed in an artificial salt solution by the X-ray diffraction analysis of Matsubara et al. (10). As for the electrophysiological properties of the myocardial cell, Rosen and his colleagues (11) have shown the similarity of the membrane action potentials of canine cardiac Purkinje fibers in Tyrode's solution and in arterial blood. Thus, it is obvious that the blood-perfused excited...
skeletal muscle is a suitable preparation for studying muscle physiology and pharmacology.

In the present study, the potassium-contracture of the excised skeletal muscle preparation of the dog was investigated in further detail and compared with the contracture induced with infusion of caffeine.

Materials and Methods

Blood-perfused, excised diaphragm preparations from dogs were used in this study, and detailed description of the preparation has already been published (8). In brief, a hemi-diaphragm, together with the supplying artery, was excised from a mongrel dog anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and the muscle was perfused with arterial blood from another anesthetized dog (donor dog) through the artery at a constant perfusion pressure of 100 mmHg by means of a peristaltic pump (Harvard Apparatus, Model 1215). Blood coagulation was prevented by sodium heparin (500 U/kg). The muscle preparation was bathed in venous blood flowing out of the muscle in a water-jacket warmed at 37 °C. The blood overflowing from the water-jacket was returned to the donor dog intravenously by gravity. Tension of the muscle stretched with a weight of 20.0 g was measured isometrically with a strain-gauge (Nihon Kohden, SB-1T) attached to the central tendinous part of the diaphragm. Blood flow through the preparation was measured with an electromagnetic flowmeter (Nihon Kohden, MF-26). Recordings were made on an ink-writing oscillograph (Nihon Kohden, RM-85).

Seventeen mongrel dogs were used in this study, the average body weight being 9.3±1.1 kg. The average wet weight of the diaphragm muscle for the preparation was 18.2±3.1 g (N=17), and the average blood flow through the phrenic artery was 7.0±1.5 ml/min (N=17) at the beginning of the measurement.

Drugs used were diltiazem hydrochloride (Tanabe), verapamil hydrochloride (Eisai), tetracaine hydrochloride (Sigma) and caffeine (Wako). The drugs were dissolved in 0.9% saline. Drug solutions in a volume of 0.01–0.3 ml were injected close-arterially to the muscle at the rubber tubing attached to the arterial cannula. The doses given refer to the bases of the drugs.

Statistical evaluation was made according to Student’s t-test. All values were expressed as the mean±S.E.

Results

In the present study it was observed that intra-arterial injections of diltiazem (3 μg–0.3 mg), verapamil (3 μg–0.3 mg), tetracaine (30 μg–3 mg), MnCl₂ (0.1–10 mg) and CaCl₂ (0.1–10 mg) had no effect on the resting tension of the diaphragm muscle preparation. However, these drugs, except for CaCl₂, produced a dose-dependent increase in blood flow rate. CaCl₂, in lower doses (0.1–1 mg), decreased the blood flow rate only for a short period; but in higher doses (3–10 mg), this brief decrease was followed by a slight increase.

1. Time course of contracture induced by KCl infusion: Continuous intra-arterial infusion of KCl to the muscle produced a contracture with various features according to the rate of infusion as shown in Fig. 1.
Infusion of KCl at a rate of 10–20 mg/min produced a monophasic contracture in which the tension gradually increased during the infusion. KCl infusion at a rate of 30–40 mg/min produced a greater contracture which developed immediately on infusion and persisted throughout the infusion. On the other hand, infusion of KCl at a rate of 50–100 mg/min produced a biphasic contracture consisting of the initial phasic and the following sustained tonic contractures as described partially in our preceding paper (9). Figure 1 shows the time courses of the contracture of the diaphragm muscle induced by various rates of KCl infusion. The biphasic feature of potassium contracture was most obvious with infusion of 50 mg/min, of which the maximum tension development of the initial phasic contracture was 204.2±17.8 g (N=17) from a basal tension of 20.0 g; and the tension maintained as a sustained tonic contracture during the infusion was 65.7±6.3 g (N=17). Infusion at higher rates produced more prominent initial phasic contractures, but much smaller sustained contractures in the tonic phase. Blood flow rate increased with 10–20 mg/min infusion of KCl, but decreased with larger doses of KCl depending on the degree of the developed tension, probably by compression to the blood vessels.

2. Effects of diltiazem, verapamil, tetracaine, MnCl₂ and CaCl₂ on the tonic contracture induced by KCl infusion: Single intra-arterial injections of verapamil (3 μg–0.3 mg) and MnCl₂ (0.1–10 mg) into the phrenic artery produced a dose-dependent relaxation of the muscle in the state of tonic contracture induced by KCl infusion (50 mg/min). Essentially the same result was obtained with diltiazem (3 μg–0.3 mg) and tetracaine (30 μg–3 mg). However, with higher doses of diltiazem and verapamil, a slight increase in muscle tension preceded the relaxation. Blood flow through the muscle was increased by verapamil, diltiazem and MnCl₂ with the same time course as the muscle relaxation. Duration of the increase in blood flow by tetracaine was shorter than that of muscle relaxation. Typical experiments are illustrated in Fig. 2, and dose-response curves for the relaxation of the muscle to diltiazem, verapamil, tetracaine and MnCl₂ are shown in Fig. 3. The dose-response curves to the four drugs were all parallel. The curves

![Fig. 2. Effects of CaCl₂, diltiazem, verapamil, MnCl₂ and tetracaine on the tension during sustained contracture induced by KCl infusion (50 mg/min) in the isolated, blood-perfused canine diaphragm preparation. All drugs were injected close-arterially to the muscle.](image)

![Fig. 3. Dose-response curves for CaCl₂, diltiazem, verapamil, MnCl₂ and tetracaine to change the tension during the tonic phase of contracture induced by KCI infusion. Each point represents the mean of five to eight determinations. Vertical bars indicate standard errors of means.](image)
showed that diltiazem and verapamil were equipotent, and these two drugs were about 10 and 30 times more potent than tetracaine and MnCl₂ on a weight basis.

In contrast to the four relaxant drugs, CaCl₂ (0.1–10 mg) produced a contractile response in a dose-dependent manner (Figs. 2 and 3). With 10 mg of CaCl₂, the peak tension development amounted to 176.6 ± 12.7% (N=7) of the tension before the injection. Duration of the vascular response to CaCl₂ was shorter than that of muscle contraction.

3. Effects of diltiazem, verapamil, tetracaine, MnCl₂ and CaCl₂ on the tonic contracture induced by caffeine infusion: Continuous intra-arterial infusion of caffeine (20 mg/min) produced a contracture in which tension slowly increased to 61.1 ± 3.6 g (N=13) from a basal tension of 20.0 g and was maintained throughout the infusion. Single intra-arterial injections of diltiazem (3 μg–0.3 mg) and verapamil (3 μg–0.3 mg) to the sustained caffeine-contracture produced no relaxation, while with higher doses of these drugs, slight increases in muscle tension were observed in a manner similar to those shown in the potassium-contracture. A single injection of MnCl₂ (0.1–10 mg) produced a slight relaxation in the tonic contracture induced by caffeine infusion, though MnCl₂ was much less effective against caffeine-contracture than against potassium-contracture. CaCl₂ (0.1–10 mg) produced relaxation of the tonic contracture induced with caffeine, although CaCl₂ was less potent than MnCl₂. On the other hand, single intra-arterial injection of tetracaine (30 μg–3 mg) produced a dose-dependent relaxation in the tonic contracture induced by caffeine infusion. These dose levels of tetracaine are almost equivalent to those which produce the relaxation on potassium-contracture. Typical experiments are illustrated in Fig. 4, and the dose-response curves for changes of the muscle tension in caffeine-contracture by the five drugs are shown in Fig. 5.

Discussion

It was demonstrated that infusion of KCl produced a contracture in the diaphragm preparation of the dog. The features of the tension development and maintenance were different according to the dose of KCl. Infusion of KCl at a rate of 50–100 mg/min produced a biphasic contracture, an initial fast transient larger contracture and a following sustained smaller contracture, as shown in our previous report (9). This rate of KCl infusion roughly makes a 125–250 mM
concentration of potassium in the cell environment. It is in accord with the observation by Dulhunty (5) that 224 mM potassium produced a biphasic contracture in excised mouse limb muscle. According to our previous results, the sustained contracture induced by 100 mg/min of KCl infusion was not influenced by tetrodotoxin, a selective inhibitor of the fast channel, but was effectively relaxed by verapamil, an inhibitor of the slow channel (9). It was not a secondary effect of the vasodilatation by verapamil since the developed tension was not affected so promptly by blood flow changes produced by changing the perfusion pressure. Thus, we have proposed that the slow channel of the skeletal muscle of the dog is activated with membrane depolarization produced by potassium infusion, and calcium influx through this mechanism is essential to the maintenance of the tonic potassium-contracture (9). In the present study, it was demonstrated that diltiazem, another calcium antagonist (12), also produced a dose-dependent relaxation of the tonic potassium-contracture in a similar manner to verapamil and MnCl2. The dose levels of verapamil, diltiazem and MnCl2 are equivalent to those for the negative inotropic effect on the cardiac muscle of the dog tested in excised cardiac papillary muscle preparations perfused with blood (13, 14). Therefore, the present results confirm that the entry of extracellular calcium ions through the activated slow-channels has an essential role in the maintenance of potassium-induced tonic contracture of skeletal muscle. This conclusion is further supported by the fact that CaCl2 injected in the state of tonic potassium-contracture produced a dose-dependent contractile response. Since CaCl2 injections to the resting muscle produced no change in the muscle tension (9), the potassium infusion has increased the availability of extracellular calcium for the contractile machinery of the diaphragm muscle cells.

On the other hand, diltiazem and verapamil had no effect on the tonic contracture induced by caffeine infusion. It has been established that caffeine elicits muscle contracture by different mechanisms from that by high potassium, namely, caffeine induces contracture by directly stimulating the sarcoplasmic reticulum to increase the free calcium level in the sarcoplasm (15). It has been known that caffeine may have another site of action that is different from the sarcoplasmic reticulum, probably on the coupling mechanism between the transverse tubular system and terminal cisternae (16). Since manganese and calcium ions are known to stabilize the mechanical activity caused by caffeine, they inhibit caffeine contracture possibly by acting on the coupling described above (17, 18). In the present study, both MnCl2 and CaCl2 produced relaxation of the contracture induced by caffeine, although to only slight extents.

Tetracaine was equally effective in producing the relaxation of tonic contractures induced by potassium and by caffeine. It is well established that tetracaine inhibits the calcium-induced calcium release mechanism (15). This drug also inhibits the slow inward current in mammalian Purkinje fiber (19) and in frog atrial muscle (20). It seems most likely, therefore, that the effect of tetracaine is ascribed to inhibition of both the calcium influx through the slow channel and the release of calcium from the sarcoplasmic reticulum.

As for the initial fast contracture, we have not come to a conclusion for the time being. Verapamil administered prior to KCl increased the fast contracture. Details of this study will be published elsewhere (21).

In addition, the small increase in the tension induced by high doses of verapamil and diltiazem is worth considering.

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