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PRENYLAMINE-INDUCED CONTRACTURE OF FROG SKELETAL MUSCLE

E.B. KIRSTEN & KAREN C. LUSTIG

Department of Pharmacology, Columbia College of Physicians & Surgeons, New York, New York 10032, U.S.A.

1 Experiments were performed to determine the influence of prenylamine on excitation-contraction coupling in frog sartorius muscle.

2 Prenylamine (0.2–1.0 mM) produced a biphasic contracture in skeletal muscle characterized by an initial phasic and subsequent tonic contracture.

3 Neither dantrolene nor procaine blocked the prenylamine-induced contracture. Pretreatment with 100 mM K^+ blocked the phasic but not the tonic component of the prenylamine contracture.

4 Prenylamine produced a sustained increase in ^{45}Ca efflux at all concentrations that produce contracture. These concentrations of prenylamine also depressed the action potential, muscle twitch and resting potential.

5 Low concentrations of prenylamine (0.05 mM) which produced neither contracture, ^{45}Ca efflux nor ^{45}Ca influx, depressed the action potential, muscle twitch and K^+ contracture.

6 The results suggest that prenylamine not only alters calcium mobility but also membrane permeability to other ions.

Introduction

Prenylamine (*N*-{3′-phenylpropyl-(2′)}-1,1-diphenyl-propyl-(3) amine; Segontin) is one of a group of compounds which have been designated as organic calcium antagonists. At least two of these compounds, verapamil and prenylamine, have clinically useful cardiac antiarrhythmic properties which have been attributed to an action on membrane calcium conductance (Singh & Vaughan-Williams, 1972; Rosen, Ilvento, Gelband & Merker, 1974; Rosen, Wit & Hoffman, 1975). Several lines of evidence have indicated that these agents have rather specific effects on calcium mobility. First, the results of studies on cardiac atrial (Haas, Kern, Benninger & Einwächter, 1975) and ventricular muscle fibres (Kohlhardt, Bauer, Krause & Fleckenstein, 1972), cardiac Purkinje fibres (Cranefield, Aronson & Wit, 1974) and fibres in the sinoatrial and atrioventricular nodes (Wit & Cranefield, 1974) are consistent with the observation that these agents depress a slow inward calcium current. Secondly, both verapamil and its methoxy-derivative D_{600} block a second, delayed component of calcium entry in squid axon (Baker, Meves & Ridgway, 1973). Thirdly, these agents have been shown to block excitation-contraction coupling in cardiac muscle (Fleckenstein, Tritthart, Fleckenstein, Herbst & Grün, 1969), smooth muscle (Haeusler, 1972) and skeletal muscle (Bondi, Kirsten & Hofmann, 1974). The apparent specificity with which these agents interfere with an inward flux of calcium ions, prompted us to investigate the effects of prenylamine on skeletal muscle.

Over the past few years, a considerable body of evidence has accumulated showing that activation of skeletal muscle proteins depends upon the release of calcium from the sarcoplasmic reticulum (Winegrad, 1970; Weber & Murray, 1973). However, the precise mechanism whereby depolarization of the transverse tubular (T-tubules) system is coupled to intracellular release of calcium is unknown. Two proposals for the coupling of sarcolemma excitation to the interaction of actin and myosin have been advanced. One suggestion (Bianchi & Bolton, 1967) involved the inward movement of a proposed 'trigger' calcium, located in the T-tubules and mobilized by T-tubular depolarization. This fraction of 'trigger' calcium regeneratively releases activator calcium from the sarcoplasmic reticulum and contraction ensues. An alternative possibility (Sandow, 1973; Podolsky, 1975; Sandow, Krishna, Pagala & Sphicas, 1975) involves an initial release of sarcoplasmic reticulum calcium caused by T-tubular depolarization and a subsequent regenerative release of activator calcium. The purpose of this investigation was to consider the effects of prenylamine on skeletal muscle with particular attention focused on the cellular calcium compartments with which prenylamine interacts. By

studying several organic calcium antagonists, a variety of pharmacological actions on skeletal muscle may be revealed thereby providing additional clues to the coupling process described above.

Methods

Sartorius muscles were dissected from pithed frogs (*Rana pipiens*) during all seasons of the year. After dissection, the muscles were equilibrated for 30–60 min at ambient temperature (22–24°C) in oxygenated Ringer solution (normal Ringer solution) containing (mM): NaCl 110.8, KCl 2.0, $CaCl_2$ 1.8, NaH_2PO_4 0.1, $NaHCO_3$ 2.02 and tris (hydroxymethyl)aminomethane 0.5 buffer adjusted to pH 7.2. Calcium-free Ringer was prepared without the addition of $CaCl_2$. Prenylamine lactate was dissolved in 100% ethanol to prepare a stock solution of 0.2 M and diluted 1:200 to 1:4000 in Ringer solution to prepare the final concentrations. The final ethanol concentrations never exceeded 0.5% (v/v). Control studies indicated that this concentration had no effect on the electrically-evoked muscle twitch. Solutions containing high concentrations of K^+ were prepared by an equimolar substitution of K^+-isethionic acid for the NaCl in the Ringer solution or Ringer solution containing prenylamine. In studies involving a second agent (i.e., dantrolene, procaine or caffeine), the test agent was added directly to the Ringer solution before use, and if necessary the pH was readjusted to 7.2 with HCl or NaOH.

Electrical and mechanical response studies

The excised sartorius muscle was mounted horizontally in an acrylic plastic bath under a tension of approximately 2 grams. During the 30–60 min equilibration period, the muscle was subjected to direct twitch and tetanic stimulation. After the equilibration period, the Ringer solution was changed for one containing prenylamine. In studies involving pretreatment with dantrolene or procaine, the muscle was allowed to equilibrate with this agent for 30 min before the addition of prenylamine. In all experiments involving a second agent, paired sartorius muscles were used.

Isometric mechanical responses due to electrical stimulation or to contracture-inducing agents were measured with a Universal transducing cell (UC-4: Statham Inc.) with an attached load cell (Statham Inc), amplified and displayed on a polygraph. The areas under the tension curves were measured with a compensating polar planimeter (Keuffel and Esser). Supramaximal twitches (stimulus duration, 2.0 ms; frequency, 1 pulse/20 s) or fused tetani (200 ms train; duration 1 ms; frequency 500 Hz) were produced by field stimulation as previously described (Ross & Brust, 1968). (+)-Tubocurarine (70 μM) was added to the Ringer solution to block responses to acetylchóline released by electrical stimulation of nerve terminals.

The method for recording resting potentials and action potentials has been described previously (Kirsten & Kuperman, 1970a; Lustig & Kirsten, 1974). Sartorius muscles were mounted dorsal side upward and at rest length in an acrylic plastic bath. Transmembrane potentials were recorded by micropipettes (10–20 MΩ) filled with 3 M KCl and with tip potentials less than 5 mV. The micropipettes were coupled by a 3 M KCl bridge to a high impedance amplifier with capacitance feedback compensation (Bioelectric Inst., NF-1) and monitored on a storage oscilloscope. Several control impalements were made in each muscle before the Ringer solution was changed for one containing prenylamine.

In some experiments, the extracellular action potential and isometric twitch were recorded simultaneously. In these experiments, electrical stimuli (30–60 V) were provided by 0.15 ms pulses at a frequency of 1 pulse/s through a bipolar electrode (David Kopf Inst. SNEX-100). Stimuli were kept submaximal so that only superficial fibres were excited, preventing gross muscle movement which would interfere with consistency in recording. Extracellular action potentials were recorded differentially through teflon-coated bipolar silver wire electrodes (0.010 inch dia.), amplified by a bandpass amplifier (Ortec Inc., 4660), displayed on an oscilloscope and photographed.

Radioisotope studies

The methods used for determining the uptake and efflux of ^{45}Ca (New England Nuclear Corp.) from sartorii were similar to those reported previously (Kirsten & Kuperman, 1970a; Lustig & Kirsten, 1974). In studies measuring ^{45}Ca uptake, one muscle was exposed for 15 min to ^{45}Ca-Ringer (4 μCi/ml) while its contralateral mate was exposed to ^{45}Ca-Ringer to which prenylamine (0.05 mM) was added. After ^{45}Ca exposure and blotting and rinsing with Ringer solution, the muscles were transferred consecutively to seven test tubes, each containing 3 ml of Ringer solution. The solutions bathing the muscle were changed and collected at 2, 5, 10, 15, 30, 60 and 90 min to wash out the extracellular calcium. At the conclusion of the washout period, the muscles were dissolved in acid and the radioactivity of the residue counted in a liquid scintillation spectrometer. After correction for decay and quenching, the data were expressed in terms of calcium influx. The ^{45}Ca space (ml per g) of the muscles multiplied by the calcium concentration of the Ringer solution (μmol per ml) determines the calcium uptake. The influx was obtained by dividing the calcium uptake (μmol per g)

by the estimated surface area of the fibres, reported as 300 cm^2/g (Bianchi & Shanes, 1959). The data were corrected for loss of ^{45}Ca from the slow compartment (Shanes & Bianchi, 1959) during the 90 min washout by using a correction factor of 1.42 (Bianchi, 1961).

Further ^{45}Ca uptake measurements were performed during K^+ depolarization. ^{45}Ca uptake was studied from paired muscles exposed for 3 min to ^{45}Ca-Ringer solution and then exposed for 2 min to ^{45}Ca-Ringer substituted with 100 mM K^+-isethionic acid in the presence (experimental) or absence (control) of prenylamine (0.05 mM). Following exposure, the residual ^{45}Ca content was determined as described previously.

Prenylamine-induced changes in the extracellular space were determined by using [^{14}C]-sucrose (4.3 mCi/mmol; New England Nuclear). After equilibration in oxygenated Ringer solution, paired sartorius muscles were transferred for a period of 30 min to Ringer solution (control) or Ringer solution (experimental) to which prenylamine was added. Directly following this treatment, the muscles were exposed for 30 min to the same solutions containing tracer amounts of [^{14}C]-sucrose. After a final blotting, the muscles were weighed and dissolved overnight in HCl. Samples of the tissue solvate were counted for [^{14}C]-sucrose as described above. Sucrose space was calculated as the ratio of the counts per min per g muscle (wet wt.) to counts per min per ml of the [^{14}C]-sucrose Ringer solution.

The radiocalcium procedures employed for the efflux data have been described previously (Kirsten & Kuperman, 1970a; Lustig & Kirsten, 1974). Paired sartorius muscles with their tendons removed, were loaded with ^{45}Ca by soaking for 3 h at ambient temperature in ^{45}Ca Ringer (4 μCi/ml). After loading, the muscles were rinsed with 20 ml of Ringer solution and gently blotted. The muscles were then placed in a chamber containing 3 ml Ringer solution. This collection fluid was changed for 3 ml of fresh solution at 10 min intervals through the entire washout period. At each 10 min interval, 1 ml of the total 3 ml washout fluid was emptied into a polyethylene scintillation vial and 15 ml of a modified Bray's scintillation fluor added (Kirsten & Kuperman, 1970a). At certain times during the washout period (to be indicated for each experiment), a test agent was added to the collection fluid. The muscle chambers were shaken during the entire washout and at no time during the washout period were the muscles removed from the chamber.

At the completion of 180 min of washout, the muscles were removed from the chamber, lightly blotted, and placed in silica crucibles. The muscles were dissolved in HCl overnight and aliquots of the tissue solvate were prepared for counting. The samples obtained at each 10 min collection period and the tissue solvate were counted in a liquid scintillation spectrometer. After correction for decay and quenching, the data for all experiments were expressed as rate coefficient curves. The rate coefficient, expressed in units of percent per min, is defined as the percentage of the average radioactivity in the muscle during the time of collection that has emerged per min of the collection period (Shanes & Bianchi, 1959). In some experiments, a relative rate coefficient curve was plotted. For these curves, the individual rate coefficients determined between 120 and 150 min of washout were averaged and taken as equal to 100%.

Comparisons between paired muscles were made with the use of Student's t test for paired comparisons. Differences were considered statistically significant at $P < 0.05$.

Drugs

Prenylamine lactate was kindly supplied by Hoechst Pharmaceutical Co. and dantrolene by Eaton Labs of the Norwich Pharmacal. Co. Caffeine base, disodium edetate (Na_2EDTA) and procaine hydrochloride were obtained from Sigma Chem. Co. (+)-Tubocurarine was purchased from Organon Inc and K^+-isethionic acid from Eastman Chemical (Rochester, N.Y.).

Results

Prenylamine-induced contracture

The response of frog sartorius muscle to high concentrations (0.2–1.0 mM) of prenylamine was characterized by a biphasic contracture as shown in Figure 1a. The peak tension produced during the initial phasic and subsequent tonic contracture (second phase) increased with prenylamine concentration. Relaxation rate was faster with higher concentrations and relaxation to resting levels occurred with the drug still present in the bath. Figure 1b shows a plot of the peak tension during the contracture vs. the log of prenylamine concentration. The threshold concentration for a measurable response was approximately 0.1 mM. The area under the contracture curves (Figure 1c) varied in the same manner as the peak tension. The biphasic response due to 0.5 mM prenylamine was similar in contracture area to that produced by 100 mM K^+ (Putney & Bianchi, 1974).

Factors modifying the prenylamine contracture

In an attempt to dissect the two phases of prenylamine contracture, we compared control contractures in one muscle with several pretreatment regimens for its contralateral mate. Dantrolene, a peripherally acting muscle relaxant (Snyder, Davis, Bickerton & Halliday, 1967), has recently been shown to be an effective blocker of excitation-contraction coupling (Ellis & Bryant, 1972; Putney & Bianchi, 1974).

Pretreatment with dantrolene, in a concentration double (16.6 μM) that used by Putney & Bianchi

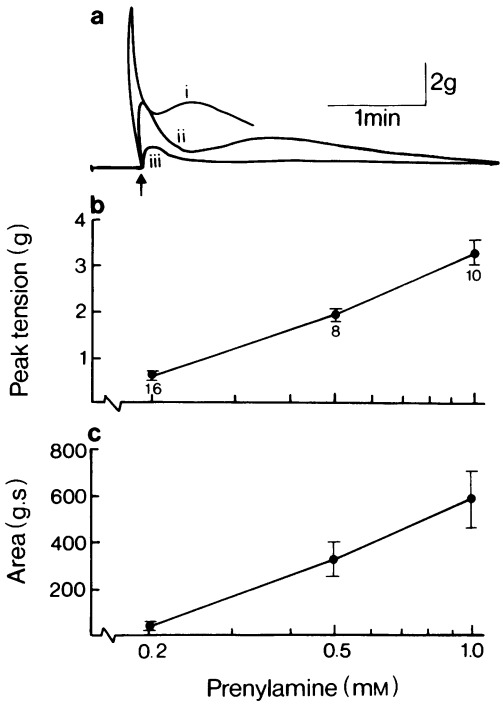

Figure 1 Prenylamine contractures in skeletal muscle. (a) Rate of isometric tension development in frog sartorii exposed to prenylamine (arrow) in concentrations of: (i) 1.0 mM; (ii) 0.5 mM; and (iii) 0.2 mM. Prenylamine produces a biphasic response, which is characterized by an initial phasic (first phase) and a subsequent tonic contracture (second phase). The tracings were recorded on a curvilinear recorder and photographically superimposed. (b) Prenylamine log dose-response curve describing the contracture of sartorius muscles exposed to 0.2 mM, 0.5 mM and 1.0 mM prenylamine. The response is expressed as peak tension developed during the first phase of contracture. The number of muscles used for each concentration is indicated and the standard error is shown. (c) Plot of area under the prenylamine contracture for the muscles exposed to prenylamine at the concentrations shown. The number of muscles used with each concentration is the same as in (b).

(1974), had no effect on prenylamine contracture ($n=6$) compared with paired muscles treated with dantrolene alone.

Procaine, in a concentration (3.67 mM) which blocks both the contracture and associated ^{45}Ca efflux induced by caffeine (Feinstein, 1963) and quinine (Suarez-Kurtz & Paumgartten, 1973), did not alter contractures produced by prenylamine.

Prenylamine-induced contractures were also studied in depolarized muscle. Potassium isethionate

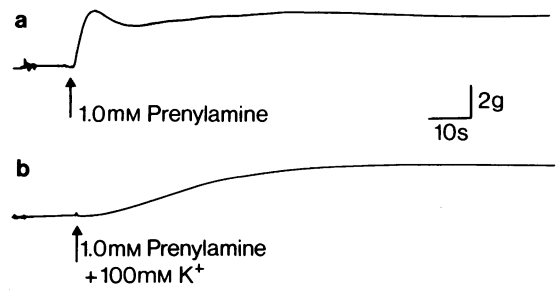

Figure 2 Effects of high K^+ on prenylamine contractures. (a) Isometric recording of a prenylamine contracture. (b) Contracture induced by prenylamine following 5 min pretreatment with 100 mM K^+ in the paired muscle. Resting tension returned to control levels before the addition of prenylamine in (b). High K^+ concentrations completely inhibit the first phase of the prenylamine contracture.

(100 mM) was employed as the depolarizing agent in order to minimize changes in cell volume and intracellular ionic concentration. The effects of K^+ pretreatment on prenylamine contractures are illustrated in a typical experiment (Figure 2). Treatment with 100 mM K^+ completely abolished the first phase of the contracture but did not alter the second phase. The results of six experiments are summarized in Table 1. In contrast to the significant ($P<0.001$) depression of the first phase of the prenylamine contracture, neither the peak tension nor the latency to peak tension of the second phase was changed.

Effects on ^{45}Ca movement

Because of the firmly established role of calcium in excitation-contraction coupling (for review, see Fuchs, 1974) and previous reports of a calcium-antagonistic effect of prenylamine (Hasselbach, Balzer & Makinose, 1968; Van der Kloot, 1973), we determined the influence of prenylamine on ^{45}Ca efflux and influx. Efflux of calcium from frog sartorius muscle can be considered as made up of two components (Shanes & Bianchi, 1959). The fast calcium component represents extracellular and loosely bound calcium; the slow component is considered to reflect calcium release from intracellular sites, presumably the sarcoplasmic reticulum. In six experiments, prenylamine (1.0 mM) added to the muscle chamber during the slow phase of ^{45}Ca washout, produced an increase in the rate coefficient for ^{45}Ca efflux as shown in Figure 3. Prenylamine caused a sustained increase in ^{45}Ca efflux from whole muscle, an effect similar to that seen by the rigour-producing agents caffeine (Isaacson & Sandow, 1967), quinine (Isaacson, Yamaji &

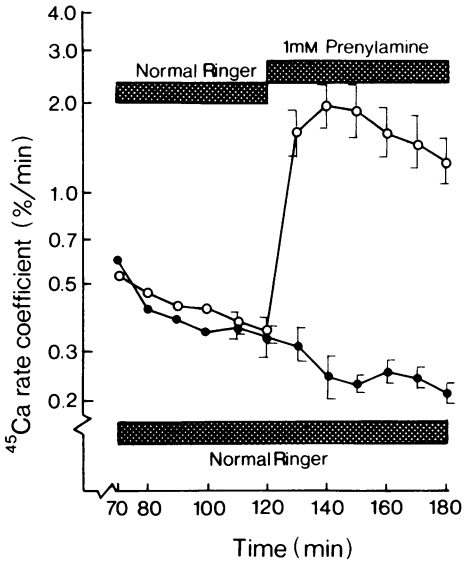

Figure 3 Average change in rate coefficient of ^{45}Ca efflux produced by 1.0 mM prenylamine (○) compared with paired untreated control muscles (●) in Ringer solution. The control muscles were washed out in Ringer solution for 180 minutes. The experimental muscles were washed out in Ringer solution to which 1.0 mM prenylamine was added from 120 to 180 minutes. Notations at the top and bottom of the graph refer to the upper (○) and lower (●) curves, respectively. Each point is the mean of six ($n=6$) separate experiments. The standard error is given for the later portion of the curves.

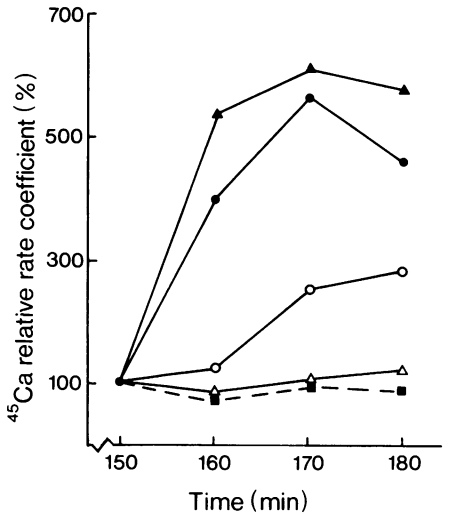

Figure 4 The effect of prenylamine on ^{45}Ca release from frog sartorius muscles. The data are plotted as a relative rate coefficient (%), where 100% is the average ^{45}Ca efflux collected from 120 to 150 min of washout. Prenylamine was added at 150 minutes. Each point is the mean of three experiments ($n=3$) and the concentrations of prenylamine used were (mM): 1.0 (▲); 0.5 (●); 0.2 (○) and 0.05 (△). No significant change ($P>0.2$) was observed for the muscles treated with 0.05 mM prenylamine and the control (■) muscles.

Sandow, 1970) and *N*-ethylmaleimide (Kirsten & Kuperman, 1970b). However, rigour development was not observed with prenylamine.

The chelator EDTA is often employed to remove external or superficial calcium from the sarcolemma, thereby allowing the effect of an agent to be studied on the release of activator calcium from the sarcoplasmic reticulum. Previous studies (Bianchi, 1965) have demonstrated that EDTA does not penetrate into the muscle fibre and hence cannot chelate sarcoplasmic reticulum calcium. After 30 min pretreatment with EDTA, prenylamine still produced a doubling in the ^{45}Ca released at the 120 min (i.e., preprenylamine) level. This increased ^{45}Ca efflux following EDTA was similar to that observed with rigour producing agents.

Release of ^{45}Ca was examined for four con-

Table 1 Effects of 100 mM K^+ on contractures due to 1.0 mM prenylamine*

	Control†	*Experimental‡*	*C–E*	*Significance*
Peak tension First phase (g)	3.13 ± 0.36	0.00	3.13	$P<0.001$
Peak tension Second phase (g)	3.38 ± 0.35	2.59 ± 0.24	0.79	NS
Latency to peak Second phase (s)	66.7 ± 12.3	61.0 ± 5.22	5.70	NS

* All values obtained for six pairs of sartorii and reported as mean ± s.e. † Control contractures produced by 1.0 mM prenylamine. ‡ Experimental muscles were pretreated for 5 min with 100 mM K^+ before adding 1.0 mM prenylamine.

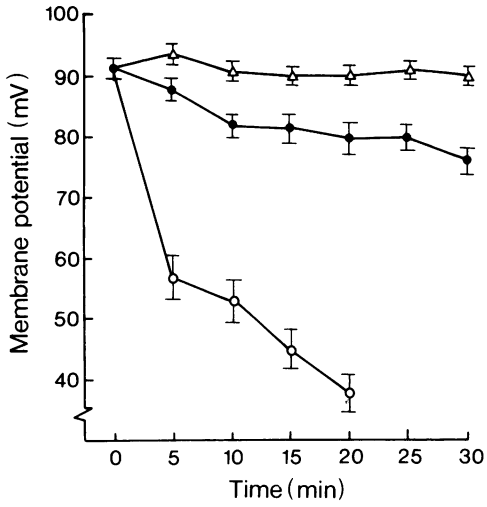

Figure 5 Transmembrane potential of surface fibres before and after the addition of prenylamine at 0.05 (△); 0.2 (●) and 1.0 (○) mM. The average control resting potential is given at zero time. The values shown are pooled averages (±s.e.) of ±2.5 min from the time indicated. Each point is the average of 25 or more impalements ($n=25$) made on three muscles. At concentrations of 0.2 mM and 1.0 mM, prenylamine causes membrane depolarization. At lower concentrations, prenylamine (0.05 mM) produces neither depolarization nor contracture.

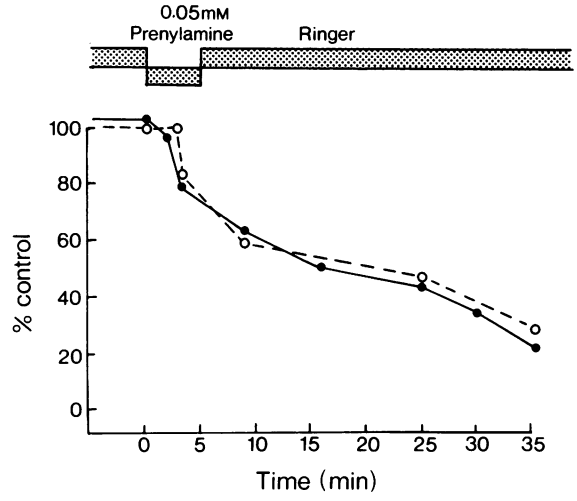

Figure 6 Typical depression of muscle action potential (○) and twitch (●) by 0.05 mM prenylamine. Inhibition of the extracellularly recorded action potential follows a time course similar to that observed for the electrically evoked muscle twitch. At 5 min, the Ringer solution was restored, although recovery was not observed. Stimulation frequency: 1 pulse/20 seconds. Higher concentrations of prenylamine produce more rapid depression of the muscle action potential and twitch.

centrations of prenylamine. We found that all concentrations of prenylamine which caused contracture also produced ^{45}Ca release (Figure 4). Low concentrations of prenylamine (i.e., 0.05 mM) produced neither ^{45}Ca release ($P>0.2$, as compared with controls) nor contracture.

Several investigators have suggested that the sustained calcium release produced by rigour-inducing concentrations of caffeine was attributable to progressive disruption of the transverse tubules and myofilaments (Huddart & Oates, 1970; Isaacson & Bárány, 1973; Lustig & Kirsten, 1974). In order to determine if the prolonged calcium release induced by prenylamine (0.2–1.0 mM) was secondary to a disruption of the sarcolemma and intracellular membrane system, we measured [^{14}C]-sucrose space in untreated and prenylamine-treated sartorius muscles. Using [^{14}C]-sucrose as a non-penetrating, non-electrolyte extracellular marker (Bozler, 1961), the average (±s.e.) sucrose space for sixteen muscles ($n=16$) was 0.26 ± 0.01 ml/gram. No significant change ($P>0.2$) in [^{14}C]-sucrose space was observed after 30 min treatment with 0.05 mM prenylamine (0.27 ± 0.01 ml/g, $n=6$) although a significant ($P<0.001$) increase in [^{14}C]-sucrose space was seen after 1.0 mM prenylamine (0.53 ± 0.02 ml/g, $n=10$). This marked increase in sucrose space may reflect a prenylamine-induced disruption of the muscle membrane at contracture producing concentrations.

In subsequent studies, we examined the effect of prenylamine (0.05 mM) on the ^{45}Ca influx in resting muscles. Experimental muscles, pretreated with prenylamine before measurement of the ^{45}Ca influx (0.075 ± 0.008 pmol Ca cm^{-2} s^{-1}), exhibited no significant difference from the control ^{45}Ca influx (0.074 ± 0.013 pmol Ca cm^{-2} s^{-1}) measured in Ringer solution.

Membrane electrical properties

As the first phase of the prenylamine contracture could be blocked by pretreatment with K^+, studies were performed to determine the transmembrane potential at three concentrations of prenylamine. At 0.2 or 1.0 mM, prenylamine caused a marked decrease in the resting potential of frog sartorius muscle (Figure 5). Resting membrane potential was not restored after extensive washing of the preparation. No significant change in resting potential was

observed after non-contracture producing concentrations (0.05 mM) of prenylamine.

Although 0.05 mM prenylamine did not depolarize sartorius muscle, it did produce a simultaneous depression of the electrically-evoked muscle twitch and extracellular action potential as illustrated in Figure 6. Recovery was not evident when the preparation was returned to normal Ringer solution.

Potassium and caffeine contractures

High K^+ and caffeine elicit muscle contracture by different mechanisms: high K^+ by causing membrane depolarization possibly through a 'trigger' calcium mechanism (Bianchi & Bolton, 1967) and caffeine by releasing bound calcium from the sarcotubular system (Isaacson & Sandow, 1967). With this in mind, studies were performed to determine the response of sartorius muscle to high K^+ or caffeine following treatment with 0.05 mM prenylamine. As shown in Table 2, prenylamine (0.05 mM) significantly reduced K^+ contracture tension (and ratio of peak tension to tetanus tension) and the ^{45}Ca uptake produced by 100 mM K^+. In contrast, this low concentration of prenylamine had no significant effect on the rigour tension produced by 10 mM caffeine. Hence, prenylamine in the appropriate concentration had some selectivity in antagonizing K^+ but not caffeine-induced contractures.

Discussion

The effects of prenylamine in a variety of systems can be explained as a direct or indirect consequence of an action on membrane calcium conductance (Fleckenstein *et al.*, 1969; Singh & Vaughan-Williams, 1972; Haas *et al.*, 1975). The possibility of prenylamine altering calcium mobility in skeletal muscle is, therefore, of considerable interest. At high concentrations (0.2–1.0 mM) prenylamine causes two phases of muscle contracture. The initial phasic contracture can be completely abolished by pretreating the muscle with K^+. Hence, the phasic aspect of the prenylamine contracture can be explained as a consequence of drug-induced depolarization of the sarcolemma and resultant increase in free calcium in the myoplasm.

A separate and distinct mechanism of action is responsible for the second, tonic contracture phase. The lack of effectiveness of either procaine (Inoue & Frank, 1962) or dantrolene (Putney & Bianchi, 1974) in antagonizing the prenylamine-induced tonic contracture would suggest that it is not mediated by an action on the sarcolemma or through activation of a 'trigger calcium' link (Bianchi & Bolton, 1967) in the excitation-contraction coupling process, respectively. An important clue to the effect of prenylamine in producing the second phase tonic contracture is that prenylamine causes a dose-related increase in ^{45}Ca efflux from whole muscle. This ^{45}Ca release is only observed at prenylamine dose levels which produce contracture and hence is similar to the ^{45}Ca release reported for rigour producing agents (Isaacson & Sandow, 1967; Isaacson *et al.*, 1970; Kirsten & Kuperman, 1970b). As found by Hasselbach *et al.* (1968), prenylamine inhibits the active uptake of calcium in preparations of isolated sarcoplasmic reticulum vesicles. Presumably, the ^{45}Ca release and the tonic contracture reflects a dose-dependent action

Table 2 Effects of 0.05 mM prenylamine on ^{45}Ca uptake and contractures induced by 100 mM K^+ or 10 mM caffeine*

	Control	*Experimental†*	*C–E*	*Significance*
100 mM K^+				
Peak tension (g)	8.56 ± 0.58	4.28 ± 0.74	4.28	$P < 0.001$
$\frac{\text{Peak tension}}{\text{Tetanus tension}}$	0.196 ± 0.01	0.092 ± 0.01	0.104	$P < 0.001$
100 mM K^+				
^{45}Ca uptake $\left(\frac{\text{nmol}}{\text{g}}\right)$	6.13 ± 0.54	4.10 ± 0.38	2.03	$P < 0.01$
10 mM Caffeine				
Peak tension (g)	23.9 ± 2.14	23.1 ± 2.13	0.8	NS
$\frac{\text{Peak tension}}{\text{Tetanus tension}}$	0.48 ± 0.04	0.47 ± 0.05	0.01	NS

* Contracture studies obtained on eight pairs of sartorii. ^{45}Ca uptake studies obtained on eleven pairs of muscles. All data are shown as mean ± s.e. † Experimental muscles were pretreated for 30 min with 0.05 mM prenylamine.

of prenylamine on the sarcoplasmic reticulum. The increase in [^{14}C]-sucrose space observed after exposure to 1.0 mM prenylamine may reflect a drug-induced disruption of subcellular structures at high concentrations as previously shown for caffeine (Huddart & Oates, 1970; Isaacson & Bárány, 1973).

In frog skeletal muscle, prenylamine induces a decrease in resting membrane potential, presumably due to an increased permeability to Na^+. Other studies with crayfish muscle (Van der Kloot, 1973) and frog skeletal muscle (Van der Kloot, Kita & Kita, 1975) indicated that prenylamine had no effects on transmembrane potential. The studies from Van der Kloot's laboratory on skeletal muscle were done at low concentrations (0.1 mM) and, hence, are similar to the results we obtained with 0.05 mM prenylamine. Unlike the decline in resting potential seen in this study with high concentrations of prenylamine, crustacean muscle is not depolarized. However, prenylamine (1.0 mM) does appear to block the calcium-dependent action potentials in this species (Van der Kloot, 1973).

In contrast to the rather diffuse actions observed at high concentrations, exposure of skeletal muscle to 0.05 mM prenylamine does not affect contracture, membrane potential, ^{45}Ca efflux or ^{45}Ca influx. The observed simultaneous depression of the action potential and twitch by prenylamine suggest a depressed excitability of the sarcolemma. This action of prenylamine is comparable to the local anaesthetic action (Lindner, 1960) and decrease in K^+ and Na^+ conductance (Van der Kloot, 1975) reported earlier. Verapamil, a papaverine derivative which reportedly also interferes with calcium mobilization and utilization (Fleckenstein, Grün, Tritthart & Byron, 1971) has also been shown to inhibit Na^+ currents in heart muscle (Shigenobu, Schneider & Sperelakis, 1974; Haas *et al.*, 1975) and have local anaesthetic properties (Singh & Vaughan-Williams, 1972; Bondi *et al.*, 1974; Van der Kloot & Kita, 1975). Hence, the calcium selectivity of these agents is lacking.

At 0.05 mM prenylamine exerts two kinds of effects on K^+-induced contractures: (1) it reduces contracture amplitude, and (2) it reduces the ^{45}Ca uptake associated with the K^+ contracture. It is likely that these effects are exerted on a membrane site rather than a deeper structure as the caffeine-induced release of activator calcium from the sarcoplasmic reticulum (Isaacson & Sandow, 1967) was not altered by prenylamine. While it is well known that K^+ contractures are dependent upon extracellular calcium (Lüttgau, 1963) it would be difficult to argue for a selective action of prenylamine on membrane calcium. More probably, prenylamine, besides altering calcium fluxes, also interferes with Na^+ and K^+ fluxes at one or more sites in the excitation-contraction coupling process.

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