A Local Anesthetic, Tetracaine, Similarly Inhibits Ag^+ and K^+ Contractures in Frog Skeletal Muscle

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Abstract To evaluate usefulness of Ag^+ contracture as a tool for elucidating the mechanism underlying the excitation-contraction coupling, the effects of tetracaine on Ag^+ contracture were compared with those on K^+ and caffeine contractures in frog skeletal muscle. Tetracaine less than $100\ \mu\text{M}$ shortened the duration of $120\ \text{mM}\ \text{K}^+$ contracture, without affecting tension amplitude. At higher concentrations of tetracaine, K^+ contracture was inhibited dose-dependently and the duration shortened. Treatment of the fibers with $20\text{--}500\ \mu\text{M}$ tetracaine for 3 min did not block the contracture induced by $25\ \text{mM}$ caffeine. Effects of tetracaine on Ag^+ contracture were similar to those on K^+ contracture. In the presence of $200\ \mu\text{M}$ tetracaine, 41% inhibition was observed in $120\ \text{mM}\ \text{K}^+$ contracture, while 43% in $100\ \mu\text{M}\ \text{Ag}^+$ contracture. Also, $200\ \mu\text{M}$ tetracaine completely inhibited the contractures induced by $40\ \text{mM}\ \text{K}^+$ or $5\ \mu\text{M}\ \text{Ag}^+$. These findings suggest that the Ag^+ may induce contractures via its action on the T/SR junction, not a direct action on the SR. Therefore, understanding the mechanism involved in the development of Ag^+ contracture would be helpful to elucidate the mechanism of excitation-contraction coupling.

Key words: Ag^+ contracture, K^+ contracture, tetracaine, caffeine.

It is widely accepted that depolarization of the transverse (T)-tubular membrane in skeletal muscle is responsible for the release of Ca^{2+} from the sarcoplasmic reticulum (SR). However, the mechanism underlying the signal transmission at the T/SR junction is poorly understood. A hypothesis that has attracted the most interest in the past decade would be charge movements within the T-tubular membrane, as reported first by SCHNEIDER and CHANDLER (1973). The experimental evidence supporting the involvement of charge movement (especially Q_y component) in the excitation-contraction coupling has increased (CAILLÉ *et al.*, 1985).

Tetracaine and dantrolene are known to interfere with the release of Ca^{2+} from the SR, probably by affecting an early step (charge movement) in the coupling process (HUANG, 1982; HUI, 1983; VERGARA and CAPUTO, 1983). We found recently that silver ion (Ag^+) produced a transient contracture in frog skeletal muscles, independent of depolarization of the surface membrane (OBA and HOTTA, 1985) and to Ca^{2+} entry across the cell membrane (OBA and HOTTA, 1987a). We have observed Ag^+ contracture to be blocked by dantrolene sodium via a mechanism similar to that related to its block of the twitch tension (OBA and HOTTA, 1987b). If the effect of tetracaine on Ag^+ contracture closely resembles that on depolarization-induced contraction, as well as in the case of dantrolene, clarifying the mechanism by which Ag^+ produces a transient contracture would help in a better understanding of the mechanism involved in the excitation-contraction coupling.

MATERIALS AND METHODS

Single fibers were dissected from the toe muscle of *Rana catesbeiana* in ice-cold Ringer's solution (115 mM NaCl, 2.5 mM KCl, 2.15 mM Na_2HPO_4 , 0.85 mM NaH_2PO_4 , and 1.8 mM CaCl_2 , pH 7.0). The methods for isometric tension measurement and detection of resting and action potentials were as previously reported (OBA *et al.*, 1983). After mounting the fiber in a chamber, the solution was replaced by a MOPS solution (115 mM NaNO_3 , 2.5 mM KNO_3 , 3 mM $\text{Mg}(\text{NO}_3)_2$, and 10 mM 3(N-morpholino)-propane sulfonic acid (MOPS), pH 7.0), in which Cl^- was replaced with NO_3^- to prevent AgCl formation. After 5 min, tetanus tension (P_0) was measured as a reference by electrical stimulation (0.2 ms duration, 50 Hz for 1 s). Following exposure of single fibers to various concentrations of tetracaine (pH adjusted to 7.0, free base, Sigma Chem. Co.) for 3 min, K^+ (40 or 120 mM) or Ag^+ (5 or 100 μM) was applied to fibers to check the effect of tetracaine on each contracture. The K^+ concentrations were increased by replacing Na^+ by K^+ in the MOPS solution. The effect of tetracaine was estimated on the basis of the peak tension induced by Ag^+ or K^+ . The amount of Ca^{2+} retained in the SR after the contracture was measured by application of 25 mM caffeine to the fiber after complete reversion of the contracture to the resting level. In experiments on K^+ contracture, 10^{-6} M tetrodotoxin was applied to the fiber together with tetracaine. In some experiments the single fiber was stimulated at a frequency of 0.1 Hz to check the effect of tetracaine on twitch tension.

The effect of tetracaine on the caffeine contracture was also studied to determine whether the inhibitory action of tetracaine on the K^+ and Ag^+ contractures was due to a direct action on the SR. After exposing the fiber to tetracaine (20 to 500 μM) for 3 min, the contracture induced by 25 mM caffeine was checked in the presence of tetracaine.

Resting and action potentials were detected in small bundles of muscle fibers (about 15 fibers) during a period of 3–5 min after immersing in various concentrations of tetracaine, using a conventional glass microelectrode. The fiber

excitation was achieved by application of a suprathreshold depolarizing pulse through a pair of platinum electrodes.

All experiments were performed at room temperature (20–24°C).

RESULTS

Effects of tetracaine on resting and action potentials

Alterations in resting membrane potential affect the amplitude of K^+ or Ag^+ contracture, since the fibers are immobilized by pretreatment with a high K^+ solution in which membranes are depolarized ("mechanical inactivation") (OBA and HOTTA, 1985). To provide a background for the description of the effects of tetracaine on K^+ and Ag^+ contractures, the effect of tetracaine on membrane potentials in the MOPS solution were measured first. Exposure of the fibers to 20 μM tetracaine for 3–5 min elicited no significant change in the resting membrane potential ($-97.8 \pm 5.7 \text{ mV}$, mean $\pm$ S.D., $n=10$) from controls ($-99.6 \pm 5.9 \text{ mV}$, $n=10$). Nine out of 10 fibers showed no action potential in response to a large

Fig. 1. Ag^+ -induced tension development in different single fibers pretreated with various concentrations of tetracaine for 3 min. Contractures evoked by 5 μM (A) and 100 μM (D) of Ag^+ without tetracaine were $0.65 \times P_0$ and $0.81 \times P_0$, respectively, where P_0 indicates the maximum tetanus tension (0.2 ms pulse duration, 50 Hz for 1 s) obtained in a control solution. Twitch tensions were inhibited rapidly following exposure of a fiber to 20 μM tetracaine (arrows in B), while 5 μM Ag^+ -induced contracture was not. Further increase in tetracaine to 100 μM led to a large reduction ($0.21 \times P_0$) of contracture produced by 5 μM Ag^+ (C). Note that a considerable tension is obtained in response to 100 μM Ag^+ even in the presence of 200 μM tetracaine, although the duration shortened (E). Calibration: vertical bar 1 mN, horizontal bar 30 s.

intensity of depolarizing pulse. When tetracaine was washed out for 20 min, all fibers recovered their responses to electrical stimulation. In all of 14 fibers examined, 50 or 500 μM tetracaine completely blocked twitch tension development within 30 s after application, although resting membrane potential was not decreased markedly by the drug (-96.8 ± 3.5 mV, $n=6$, at 50 μM ; -95.6 ± 5.0 mV, $n=8$, at 500 μM).

Inhibition of Ag^+ and K^+ contractures by tetracaine

The effect of tetracaine on the Ag^+ contracture was evaluated using 5 and 100 μM of Ag^+ . In intact fibers immersed in a MOPS solution, the peak tension induced by 5 and 100 μM Ag^+ were $0.64 \times P_0 \pm 0.13$ (mean $\pm$ S.D., $n=5$) and $0.86 \times P_0 \pm 0.07$ ($n=10$), respectively, as seen in Fig. 1A and D, thereby indicating that 100 μM Ag^+ produced near the maximum contraction. P_0 represents tetanus tension observed in MOPS solution immediately before application of Ag^+ . Exposure of the fiber to 20 μM tetracaine for 3 min did not affect the 5 μM Ag^+ contracture ($0.62 \times P_0 \pm 0.10$, $n=5$, Fig. 2), whereas the twitch tension was blocked (Fig. 1B). In the solution containing 50 or 100 μM tetracaine, the peak tension induced by 5 μM Ag^+ decreased to 69 or 27% of control values ($0.44 \times P_0 \pm 0.25$ at 50 μM and $0.17 \times P_0 \pm 0.12$ at 100 μM) (Figs. 1C and 2). Increase in concentration of tetracaine to 200 μM led to the development of a small tension in 2 out of 5 fibers examined, and

Fig. 2. Effect of tetracaine on K^+ (120 mM, $\circ$)-, Ag^+ (5 μM , $\bullet$; 100 μM , $\times$)-, and caffeine (25 mM, $\square$)-induced contractures. Each contraction is normalized to the tetanus tension (P_0) obtained in a control solution before exposure of the fiber to tetracaine. The fiber was pretreated with tetracaine for 3 min. The abscissa gives concentrations on a log scale. The curves were drawn by eye. Note a marked similarity on inhibitory action of tetracaine between 120 mM K^+ and 100 μM Ag^+ contractures. No contracture was produced by Ag^+ (3 fibers for 5 μM and 6 for 100 μM) in the presence of 500 μM tetracaine. Data shown in Figs. 1 and 3 are included.

the remaining 3 fibers did not contract. The relationship between the relative tension induced by $5 \mu\text{M}$ Ag^+ and tetracaine concentration is illustrated in Fig. 2, $\bullet$. All fibers studied in the presence of $200 \mu\text{M}$ tetracaine, on the other hand, produced a considerable tension in response to $100 \mu\text{M}$ Ag^+ ($0.49 \times P_0 \pm 0.18$, $n=5$) (Fig. 2, $\times$), although the duration of contracture became short (Fig. 1E). Therefore, the inhibitory effect of tetracaine depended on the concentration of Ag^+ or the ability to develop the contracture. The $100 \mu\text{M}$ Ag^+ contracture was completely inhibited by $500 \mu\text{M}$ tetracaine (Fig. 2, $\times$).

It is known that an abrupt exposure of the fiber to 120 mM K^+ rapidly depolarizes the membrane potential and a transient contracture with almost the maximum amplitude ensues (HODGKIN and HOROWICZ, 1960). In the MOPS solution used here, the fibers were depolarized to $-5.0 \pm 4 \text{ mV}$ ($n=5$) in response to 120 mM K^+ from $-99.6 \pm 5.9 \text{ mV}$ ($n=10$) of controls, resulting in almost the maximum tension development ($0.97 \times P_0 \pm 0.06$, $n=5$, see Fig. 2, $\circ$). Figure 3 shows that tetracaine at concentration of $20 \mu\text{M}$ (E), $100 \mu\text{M}$ (B), or $200 \mu\text{M}$ (C) shortens dose-dependently the duration of contracture induced by 120 mM K^+ , and at higher concentration inhibits the peak tension. At $20 \mu\text{M}$, the K^+ contracture was not inhibited ($0.97 \times P_0 \pm 0.12$, $n=5$). However, 41% inhibition of the peak tension

Fig. 3. Contractures caused by 120 mM K^+ in a single fiber pretreated with $20 \mu\text{M}$ (E), $100 \mu\text{M}$ (B), or $200 \mu\text{M}$ (C) of tetracaine for 3 min. A and D indicate K^+ contractures observed in a control solution. The two lower traces (D and E) are observed sequentially without interruption after evaluating the effect of $200 \mu\text{M}$ tetracaine K^+ contracture (C). After the K^+ contracture, the fiber was placed in a normal Ringer's solution without any drug (NR) for 15 min, to recover from the K^+ inactivation and to wash out tetracaine. P_0 represents the tetanus tension obtained in a control solution. See Fig. 1 for calibration.

Fig. 4. Complete inhibition of 40 mM K^+ contracture ($0.59 \times P_0$) by 200 μ M tetracaine in a single fiber. The fiber responds again to 120 mM K^+ after removal of tetracaine. Other notations are as in Fig. 3.

was observed after the pretreatment with 200 μ M tetracaine for 3 min ($0.57 \times P_0 \pm 0.01$, $n=5$). Such effects of tetracaine were reversible, as seen in Fig. 3D, in which replacement of tetracaine by normal MOPS solution produced a contracture upon 120 mM K^+ similar to that in control (Fig. 3A). Further increase in the concentration of tetracaine to 500 μ M showed almost complete inhibition of K^+ contracture. Only one out of 7 fibers examined developed a small contracture in response to 120 mM K^+ ($0.13 \times P_0$). Figure 2, $\circ$ summarizes the relationship between the degree of inhibition in K^+ contracture and tetracaine concentration. The effect of 200 μ M tetracaine on contracture induced by 40 mM K^+ was studied to compare with the findings obtained by 5 μ M Ag^+ , because both muscle activators elicited the similar amplitude of tension in MOPS solution ($0.64 \times P_0$ at 5 μ M Ag^+ and $0.59 \times P_0$ at 40 mM K^+). This concentration of K^+ did never elicit any contraction in the presence of 200 μ M tetracaine, in all fibers used ($n=6$). A typical trace is shown in Fig. 4. After washing out tetracaine, the fiber could again respond to 120 mM K^+ .

Effect of tetracaine on caffeine-induced contracture

As shown in Fig. 2, $\square$, peak tensions induced by 25 mM caffeine were not inhibited by treating the fiber with tetracaine over a range of 20 to 500 μ M. However, the rate of rise of caffeine contracture was decreased by tetracaine, as evidenced by the time (T_{50}) required to reach 50% amplitude of the peak tension after application of 25 mM caffeine. In the presence of 20 μ M tetracaine, the T_{50} was 0.58 ± 0.23 s ($n=5$), corresponding to that without tetracaine (0.64 ± 0.17 s, $n=5$). Increase in the concentration of the drug prolonged T_{50} dose-dependently (0.94 ± 0.43 s, $n=5$, at 50 μ M; 1.40 ± 0.81 s, $n=4$, at 100 μ M; 1.82 ± 1.12 s, $n=5$, at 200 μ M; 5.01 ± 2.24 s, $n=10$, at 500 μ M tetracaine).

DISCUSSION

Since Ag^+ is not physiologically important in intact skeletal muscle, it would be worthwhile evaluating its usefulness as a tool for studying the mechanism(s) underlying the excitation-contraction coupling. Thus, the effects of tetracaine on “depolarization (K^+)”-induced contracture were compared with those on Ag^+ -induced one. As shown in Fig. 3, tetracaine had two different actions on K^+ contracture, depending on its concentration. Low concentrations (less than $100\text{ }\mu\text{M}$) of tetracaine shortened the duration of contracture, without affecting tension amplitude, while the contracture was abolished in the presence of higher concentrations ($200\text{ }\mu\text{M}$ in the case of 40 mM K^+ contracture and $500\text{ }\mu\text{M}$ in 120 mM K^+ contracture, thereby indicating a large shift in the contractile threshold by tetracaine). The local anesthetic also blocked the action potential, with little effect on the resting membrane potential. Such effects of tetracaine on K^+ contracture are consistent with the findings reported already (LÜTTGAU and OETLIKER, 1968; HELLENBRECHT, 1971; CAPUTO, 1972, 1976; ALMERS and BEST, 1976; CAPUTO and BOLAÑOS, 1987). On the peak amplitude and the duration of Ag^+ contracture and on the contractile threshold for Ag^+ contracture development, tetracaine showed comparable effects with those on K^+ contracture (Figs. 1 and 2). This marked similarity between the inhibitory action of tetracaine on K^+ and Ag^+ contractures indicates that both types of contracture may be governed by the same mechanism(s).

In intact fibers, there are many possible sites of action of tetracaine to block the muscle contraction: (i) surface membrane, (ii) T-tubular system, and (iii) SR membrane. Tetracaine even in a low concentration ($20\text{ }\mu\text{M}$) blocked the action potential without affecting the resting potential, followed by no twitch tension (Fig. 1B). These results suggest the inhibition of Na^+ channels by the local anesthetic. However, both K^+ and Ag^+ contractures were not suppressed in the presence of $20\text{ }\mu\text{M}$ tetracaine, with or without $1 \times 10^{-6}\text{ M}$ tetrodotoxin, a specific Na^+ channel blocker. Thus, such an action of tetracaine on Na^+ channel located on surface membranes is not responsible for the inhibition of contracture developed by K^+ or Ag^+ .

Caffeine contracture is elicited by a release of Ca^{2+} from the SR (ENDO, 1977). A well-known consequence of tetracaine treatment is to inhibit the caffeine contracture at mM concentrations (LÜTTGAU and OETLIKER, 1968; ALMERS and BEST, 1976). In the present experiment, the rate of rise of caffeine contracture was decreased by tetracaine dose-dependently, thereby indicating the inhibition of the release of Ca^{2+} from the SR. However, this action of tetracaine is too small to account for the complete inhibition of contracture induced by $100\text{ }\mu\text{M Ag}^+$. At $500\text{ }\mu\text{M}$ tetracaine, peak amplitude of the contracture produced by 25 mM caffeine was not reduced (Fig. 2, $\square$), whereas Ag^+ contracture was completely blocked (Fig. 2, $\times$). Thus, the direct action of tetracaine on the SR plays no crucial role for the development of contracture induced by Ag^+ .

Since the first report by SCHNEIDER and CHANDLER (1973), the experimental evidence supporting the involvement of charge movement, especially a Q_y component, in the coupling between the depolarization of T-tubular membrane and muscle contraction has been substantially increased (HUANG, 1982; HUI, 1983; RAKOWSKI *et al.*, 1985; MELZER *et al.*, 1986). Caputo and his colleagues have suggested that the hump (or Q_y) component of the charge movement is responsible for a cause of the release of Ca^{2+} from the SR, because the close parallelism is between the tetracaine-sensitive Q_y component and the Ca^{2+} signal detected luminescently and the former always preceded the latter (CAPUTO *et al.*, 1979; VERGARA and CAPUTO, 1983). Dantrolene sodium, a muscle relaxant, inhibited also a Q_y component of charge movement with a marked similarity to the effects of tetracaine on charge movement (HUI, 1983). On the other hand, we found that dantrolene gave similar inhibitory effects on the twitch tension and Ag^+ contracture (OBA and HOTTA, 1987b), as well as the effects of tetracaine on K^+ and Ag^+ contractures in the present experiment. It is interesting that two drugs which are different in nature, tetracaine and dantrolene, have the same effect on a component of the charge movement, depolarization-induced contractions and Ag^+ contracture. Depolarization of the T-tubular membrane or the binding of Ag^+ with T-tubular membrane might move the charged particles to initiate the release of Ca^{2+} from the SR. The inhibition of the charge movement induced by exposure of the fiber to tetracaine or dantrolene may lead to the reduction in tension.

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