THE EFFECT OF PROCAINE ON THE URINARY BLADDER SMOOTH MUSCLE OF BULLFROGS

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Summary The effect of procaine on the bullfrog urinary bladder smooth muscle was studied with the Rapid Cooling technique, with the following results.

1. Procaine induced contracture in the bullfrog urinary bladder smooth muscle in normal Ringer's solution, but it induced a relaxing effect in isotonic potassium Ringer's solution.
2. Procaine-induced contracture was inhibited in a low pH Ringer's solution and potentiated in a high solution.
3. Mn^{++} inhibited the procaine-induced contracture, but tetrodotoxin produced no effect.
4. The bullfrog urinary bladder smooth muscle contracted in normal and potassium Ringer's solutions when the temperature of the solution was lowered rapidly from 26-28°C to 2-4°C (Rapid Cooling Contracture, RCC).
5. The RCC of the bullfrog urinary bladder in normal and potassium Ringer's solutions was potentiated by procaine, and was inhibited by Mn^{++} .
6. The mechanism of the procaine-induced contracture and the effect of Mn^{++} are discussed with respect to the origin of Ca^{++} . It is postulated that the effect of procaine on bullfrog urinary bladder smooth muscles was produced by mobilization of intracellular bound Ca^{++} , and the inhibitory effect of Mn^{++} on the procaine-induced contracture and the rapid cooling contracture may be produced by inhibition of its mobilization.

SAKAI (1962, 1965) and SAKAI *et al.* (1971) reported that frog skeletal muscle fibers treated with a subcontracture concentration of caffeine in normal Ringer's solution produced a remarkable contracture when the temperature of the solution was rapidly lowered (Rapid Cooling Contracture, RCC). The RCC was as-

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sumed to be triggered by Ca^{++} release from the sarcoplasmic reticulum and by the inhibition of Ca^{++} uptake by the reticulum.

RCC was also observed in the urinary bladder smooth muscle of toads (SAKAI and IIZUKA, 1972) and in the taenia coli, stomach, urinary bladder of guinea pigs (MAGARIBUCHI *et al.*, 1973; KURIHARA *et al.*, 1973). The RCC in the smooth muscle was attributed to Ca^{++} mobilization from its binding sites in the cell membrane and the intracellular membrane system.

On the other hand, procaine was said to inhibit a caffeine-RCC in frog skeletal muscle fiber (SAKAI, 1963) and in toad urinary bladder smooth muscle (SAKAI and IIZUKA, 1972). WEBER and HERZ (1968) also reported that procaine inhibited Ca^{++} release from the sarcoplasmic reticulum. FEINSTEIN (1966) and FEINSTEIN and PAIMRE (1969) confirmed the nonspecific inhibitory effect of procaine on the contraction of smooth muscle. They considered that this mechanism was attributed to the inhibition of Ca^{++} permeation through the cell membrane and the intracellular membrane system of smooth muscle.

However, it was found in the present experiments that procaine induced contraction of the smooth muscle preparations of the bullfrog urinary bladder in normal Ringer's solution. Furthermore, procaine did not inhibit the RCC of this preparation but potentiated it. Recently it has been observed that procaine inhibits the binding of Ca^{++} by the sarcolemma of rabbit skeletal muscle (THORPE and SEEMAN, 1971; MADEIRA and CARVALHO, 1972). Moreover, it is known that there are differences in the effects on the sarcoplasmic reticulum of charged and uncharged forms of procaine, the latter releasing Ca^{++} from the sarcoplasmic reticulum (BIANCHI and BOLTON, 1967; THORPE and SEEMAN, 1971).

The present experiment was made to study relationships between the effect of procaine and the Ca^{++} mobilization from its binding sites. The preliminary report of this study was presented at the 14th Annual Meeting of the Japanese Society of Smooth Muscle, held in 1972 in Tokyo, Japan.

METHODS

The urinary bladders of Japanese bullfrogs (*Rana catesbeiana*), weighing 150–200 g each, were used in this experiment. After pithing and bleeding, the urinary bladder divided in two lobes was removed. Rectangular tissue strips, 3–4 cm in length and 1.5–2 mm in width, were cut off from the ventral sides of the right and left lobes along the veins. These tissue strips were used as the preparations throughout in this experiment.

With silk threads, one end of the preparation was fixed to a glass rod and the other end to a mechano-transducer, and the fixed preparation was immersed in a cylindrical glass vessel containing 40 ml of Ringer's solution kept at 26–28°C. The rapid cooling of the preparation was performed by promptly replacing the vessel with another vessel containing a cold solution (2–3°C). After the preparation immersed in the vessel initiated weak rhythmic contractions, K^{+} -contrac-

ture was induced as the control experiment. Then the preparation was washed with Ringer's solution and left in the solution for about 30 min. These procedures were always performed on each preparation prior to consecutive experiments.

The mechano-transducer was a U-type strain gauge (from Shinko Communication Ind. Co., Ltd.). Muscle tension was recorded with a pen-writing oscillograph (WI-130 M, the Nihon Kohden Co., Ltd.).

Ringer's solution of the following composition was used: 111.2 mM NaCl, 1.88 mM KCl, 1.08 mM CaCl_2 , 2.38 mM NaHCO_3 , 0.083 mM NaH_2PO_4 , and 11.1 mM glucose. The solution was bubbled with a gas consisting of 97% O_2 and 3% CO_2 . In potassium Ringer's solution, all NaCl was replaced by KCl isototically, but 2.46 mM Na^+ was left as a component of the buffer. MnCl_2 was employed to investigate the effect of Mn^{++} on the preparation of bullfrog urinary bladder. When the effect of Mn^{++} was investigated, 2.5 mM Tris-HCl buffer was employed instead of NaHCO_3 and NaH_2PO_4 . Unless stated otherwise, the pH of the solution was adjusted to 7.2–7.3. When the pH of the solution was to be altered, HCl or NaOH was used for alteration, and the result was checked with Hitachi-Horiba's pH meter type M-5. The procaine-HCl employed in this experiment was a product of the Sigma Chemical Company.

This experiment was carried out from April through September 1972.

RESULTS

Procaine-induced contracture

Procaine-induced contracture of the preparation of bullfrog urinary bladder is shown in Fig. 1. The maximum peak tension of this contracture was nearly the same as that obtained by K^+ . With a low concentration of procaine, the muscle attained a maximum peak tension and gradually returned to its resting tension level, and after several minutes phasic contractions appeared periodically (Fig. 1A). The phasic contractions were greater than spontaneous rhythmic contractions observed in normal Ringer's solution and lasted longer.

Elevation of procaine concentration increased the frequency of these phasic contractions, and simultaneously produced a sustained tonic contraction. With 15 mM procaine the sustained tonic contraction appeared remarkably, and phasic and oscillatory contractions were superposed (Fig. 1B). The procaine-induced contracture lasted about an hour, and eventually phasic and oscillatory contractions disappeared leaving only the sustained tonic contraction in action. After procaine-induced contracture, the preparation was kept immersed in normal Ringer's solution for 30 min, and a subsequent experiment was performed. Without sufficient rinsing with normal Ringer's solution, the preparation could not produce appreciable contracture again.

Effect of pH on the procaine-induced contracture

Recently, BIANCHI and BOLTON (1967) and THORPE and SEEMAN (1971) re-

Fig. 1. Procaine-induced contracture of the bullfrog urinary bladder smooth muscle in normal Ringer's solution. A. Thirty minutes after the occurrence of K^+ -contracture (a), 1 mM procaine was applied for 20 min (b). Thirty minutes after the removal of the 1 mM procaine, 5 mM procaine was allowed to act for 20 min (c). Records (a, b, c) were obtained from the same preparation. B. Thirty minutes after the initiation of K^+ -contracture (a), 10 mM procaine was applied for 20 min (b). Thirty minutes after removal of the 10 mM procaine, 15 mM procaine was applied for 20 min (c). Records (a, b, c) were obtained from the same preparation. Different preparations were used in A and B. The pH of the solution was 7.2.

ported that the action of procaine on skeletal muscle fibers was different depending on the pH of the solution. Therefore the effect of pH on the procaine-induced contracture of the preparation of bullfrog urinary bladder was investigated here. As shown in Fig. 2, 15 mM procaine induced phasic contractions periodically at pH 6.5, and elicited a sustained tonic contraction at pH 8.0. In general, this tonic contraction tended to be augmented at a higher pH; the phasic contractions were conspicuous at a lower pH.

Effect of tetrodotoxin and Mn^{++} on the procaine-induced contracture

Tetrodotoxin (TTX) is known to block the Na^+ spike by inhibiting active Na^+ influx (NARAHASHI *et al.*, 1964). In smooth muscle, TTX did not block spike discharges but rather increased their frequency (KURIYAMA *et al.*, 1966). In the

Fig. 2. Effect of pH on the procaine-induced contracture. Twenty minutes after the observation of K^+ -contracture, the pH of normal Ringer's solution (pH 7.0) was changed to 6.5. Ten minutes after changing pH, 15 mM procaine (pH 6.5) was applied (b). Twenty minutes after removal of procaine, the pH of the normal Ringer's solution was elevated to 8.0, and 10 min after the elevation, 15 mM procaine (pH 8.0) was applied (c). After washing out the 15 mM procaine (pH 8.0) with normal Ringer's solution, tonic contraction gradually decreased (d). Tonic contraction was dominated at pH 8.0.

preparation of bullfrog urinary bladder the spontaneous rhythmic contractions were not suppressed by TTX. In this experiment, the procaine-induced contracture was observed even 15 min after the application of TTX, and its maximum peak tension was not changed markedly (Fig. 3).

It is known that Mn^{++} inhibits the Ca^{++} spike in the barnacle muscle, crayfish muscle, and frog ventricular muscle and also that the inhibitory effect of Mn^{++} is involved in E-C coupling of these muscles (FATT and GINSBORG, 1958; ORKAND, 1962; HAGIWARA and NAKAJIMA, 1966). Mn^{++} is also considered to participate in E-C coupling in smooth muscle, since it inhibited spike discharges and K^+ -contracture, and increased its membrane resistance (NONOMURA *et al.*, 1966; BÜLBRING and TOMITA, 1969). As shown in Fig. 4A, when the preparation was immersed in normal Ringer's solution for 30 min after the contracture was produced by 10 mM procaine for about 5 min, its spontaneous rhythmic contractions were completely inhibited by the application of 5 mM Mn^{++} . Ten minutes later reapplication of procaine did not induce contractions. The sustained tonic contraction of the procaine-induced contracture was also inhibited by the application of 5 mM Mn^{++} (Fig. 4B). When Mn^{++} was removed from the solution,

Fig. 3. Effect of TTX on the procaine-induced contracture. Twenty-five minutes after the occurrence of K⁺-contracture, TTX (10⁻⁶ g/ml) was applied to normal Ringer's solution. Fifteen minutes later, 15 mM procaine Ringer's solution containing TTX was applied for 8.5 min (b). Twenty-two minutes after the removal of the procaine, the preparation was immersed in normal Ringer's solution, and bathed for 30 min. Then, only 15 mM procaine was added to the normal Ringer's solution for 5 min (c). TTX did not inhibit the procaine-induced contracture.

this sustained tonic contraction was gradually recovered to some extent. The reapplication of Mn⁺⁺ inhibited the sustained tonic contraction again. When the concentration of Mn⁺⁺ was increased, the recovery of the tonic contraction was considerably delayed.

Effect of procaine on the RCC

A frog skeletal muscle fiber, treated with a subcontracture concentration of caffeine, produced a conspicuous contraction when subjected to a rapid cooling treatment. In the progress of our investigation, the RCC of smooth muscle was found to appear without caffeine treatment. The mechanism of RCC in smooth muscle is assumed in connection with the dissociation of bound Ca⁺⁺ of the cell membrane and the intracellular membrane system as in the case of skeletal muscle. The RCC without caffeine treatment was also observed in the preparation of bullfrog urinary bladder (Figs. 5A and 6A). The maximum peak tension of the RCC in normal Ringer's solution was about half that of the K⁺-contracture and was smaller than that of the RCC of the toad and the guinea pig urinary bladder.

Fig. 4. Effect of Mn^{++} on the procaine-induced contracture. A. Thirty minutes after initiation of K^{+} -contracture, 10 mM procaine was applied for 5 min (a). Thirty minutes after removal of the procaine, 5 mM Mn^{++} was applied, which abolished spontaneous rhythmic contractions. Ten minutes later, 10 mM procaine was applied to the Ringer's solution containing 5 mM Mn^{++} . The procaine-induced contracture was completely inhibited by the Mn^{++} . B. Thirty minutes after occurrence of K^{+} -contracture (a), 10 mM procaine was applied to normal Ringer's solution, and a procaine-induced contracture was observed. Four minutes later, 5 mM Mn^{++} was applied to the Ringer's solution containing 10 mM procaine, and the tonic contraction caused by procaine was reduced markedly. Three minutes after the removal of the Mn^{++} , the tonic contraction reappeared (b). Reapplication of Mn^{++} reduced the tonic contraction once more. Different preparations were used in A and B.

It was reported that the RCC of the skeletal muscle fiber was not affected by cocaine but was inhibited by procaine (SAKAI, 1963). It was also found that caffeine potentiated the RCC of toad urinary bladder in potassium Ringer's solution whereas procaine inhibited the potentiating effect of caffeine (SAKAI and IIZUKA, 1972). To compare with these findings, the effect of procaine on the RCC of the bullfrog urinary bladder was investigated.

As shown in Fig. 5, the RCCs without caffeine were repeatedly produced in normal Ringer's solution at 15-min intervals after the cessation of K^{+} -contracture. Occasionally the maximum peak tension of the first RCC was smaller than those of the second and third RCC, as depicted in the figure, and then the maximum peak tension of the following RCCs declined gradually. The application of 1 mM

Fig. 5. Effect of procaine on the RCC in normal Ringer's solution. A. Thirty minutes after initiation of K⁺-contracture, the RCCs were produced at 15-min intervals. Rapid lowering of the temperature of the solution from 27° to 3°C induced contraction (a). The rapid cooling at 10 min after application of 1 mM procaine potentiated the RCC (b). After the removal of the procaine (c), the RCC was remarkably reduced. Records (a, b, c) were obtained continuously from the same preparation. B. A graph obtained by plotting the maximum peak tension of the RCC in A at time intervals. ■ indicates the presence of procaine.

procaine for 10 min potentiated tension output of the RCC. With 1 mM procaine the sustained tonic contraction was not enhanced, but the RCC was potentiated. With the removal of the procaine a marked decrease of the RCC was observed. However, the addition of 10 mM procaine to normal Ringer's solution produced a sustained tonic contraction, and it exerted strong potentiating effect on the RCC in the sustained tonic contraction. The 10 mM procaine produced a stronger potentiating effect on the RCC than 1 mM procaine.

The RCC was potentiated by procaine in potassium Ringer's solution (Fig. 6). Thirty minutes after immersion in potassium Ringer's solution, the preparation produced RCCs repeatedly at 15-min intervals. When 5 mM procaine was added

Fig. 6. Effect of procaine on the RCC in potassium Ringer's solution. A. After 30-min immersion of the preparation in potassium Ringer's solution, the RCCs were observed at 15-min intervals (a). Each vertical bar is 0.4 g and each horizontal bar 1 min. Rapid fall of the temperature of the solution from 26° to 2°C induced contraction. Application of 5 mM procaine reduced the tonic response of K⁺-contracture slightly and transiently, but it gradually returned to the initial level (b). Ten minutes later, potentiation of the RCC was observed. At the same time it was found that the relaxation occurring at the recovery of the temperature was remarkable. Concomitantly with the removal of the procaine, the tonic response was increased, but gradually returned to the initial level. Also the RCC diminished soon (c). Records (a, b, c) were obtained continuously from the same preparation. B. A graph obtained by plotting the maximum peak tension of the RCC in A at time intervals. ■ indicates the presence of procaine.

to the potassium Ringer's solution the tonic response of the muscle, *i.e.*, K⁺-contracture, was reduced slightly, but returned quickly to its original level. Ten minutes after application of procaine, the RCC was potentiated and on

Fig. 7. Effect of Mn^{++} on the RCC in normal Ringer's solution. A. Thirty minutes after the initiation of K^+ -contracture, the RCCs were produced in normal Ringer's solution at 15-min intervals (a). Application of 3 mM Mn^{++} inhibited spontaneous rhythmic contractions as well as the RCC (b). Ten minutes after the removal of the Mn^{++} , the RCC still remained inhibited, but it returned after 25 min. After addition of the Mn^{++} to the solution, resting tension rose gradually throughout the experiment. — indicates initial resting tension level before application of 3 mM Mn^{++} to normal Ringer's solution. Records (a, b, c) were obtained continuously from the same preparation. B. A graph obtained by plotting the maximum peak tension of the RCC in A at time intervals. □ indicates the presence of Mn^{++} .

rewarming from 2° to $26^\circ C$ there was marked relaxation. The muscle was relaxed below the basal tension level, and gradually returned to the initial level. After removal of procaine, the tonic response of K^+ -contracture was transiently elevated, and gradually returned to its original level. Then the maximum peak tension of the RCC decreased quickly, and the marked relaxation observed on rewarming was disappeared.

Effect of Mn^{++} on the RCC

The effect of Mn^{++} on the RCC of the bullfrog urinary bladder preparation is shown in Fig. 7. After the RCCs were initiated in normal Ringer's solution, the spontaneous rhythmic contractions disappeared with the application of 3 mM Mn^{++} . Ten minutes after the application of Mn^{++} , the RCC was inhibited. Ten

Fig. 8. Effect of Mn^{++} on the RCC-potentiating effect of procaine in potassium Ringer's solution. A. After the preparation was immersed in potassium Ringer's solution for 20 min, the RCCs were observed at 15-min intervals (a). After observing the potentiating effect of 3 mM procaine on the RCC (b), 6 mM Mn^{++} was applied to the solution (c), which lowered the tonic response of K^{+} -contracture. Ten minutes later, maximum peak tension of the first RCC after the application of Mn^{++} was rather large, but during the cooling treatment, the tension did not persist, but relaxed. Thereafter the RCC was inhibited, and relaxation was induced by rapid cooling. At the same time, the tonic response was increased gradually. Records (a, b, c) were obtained continuously from the same preparation. B. A graph obtained by plotting the maximum peak tension of the RCC in A at time intervals. ■ denotes the presence of procaine, and □ the action of Mn^{++} .

minutes after the removal of the Mn^{++} , its inhibitory effect still persisted, but 25 min after the removal, the normal RCC recovered. With the increase of Mn^{++} concentration its inhibitory effect was enhanced, and with its removal the effect of the Mn^{++} persisted, and the recovery of the RCC was markedly delayed. After the application of Mn^{++} , the resting tension of the muscle preparation continued to rise slightly throughout the experiment.

Mn^{++} also produced an inhibitory effect on the RCC in the potassium Ringer's solution and inhibited the RCC-potentiating effect of procaine (Fig. 8). After 3 mM procaine potentiated the RCC in the potassium Ringer's solution, 6 mM Mn^{++} was applied to the solution. The tonic response of the K^+ -contracture was slightly reduced by Mn^{++} and then continued to increase gradually throughout the experiment. After the application of Mn^{++} , the first RCC was rather accelerated but the following RCCs were not observed. Immediate relaxation was brought about by rapid cooling. After the immediate relaxation was initiated by Mn^{++} during rapid cooling treatment, this phenomenon remained even after the removal of the Mn^{++} . The relaxing effect of the Mn^{++} during the rapid cooling treatment was observed even in the potassium Ringer's solution without procaine.

DISCUSSION

It is known that procaine blocks the action potential chiefly by inhibiting the Na^+ -carrying system of the cell membrane and reduces K^+ conductance slightly (WEIDMANN, 1955; SHANES, 1958; SHANES *et al.*, 1959; INOUE and FRANK, 1962; TAYLOR, 1959). BLAUSTEIN and GOLDMAN (1966) suggested that Ca^{++} and procaine compete in their actions on the membranous ion conductance mechanism. In a crayfish abdominal muscle fiber, procaine converted the graded response into an action potential of the all-or-none type, probably because procaine reduced K^+ permeability (OZEKI and GRUNDFEST, 1965; TAKEDA, 1967). It has recently been found in amphioxus muscle cells that procaine elicits regenerative potential changes by inhibiting K^+ conductance, subsequently elevating Ca^{++} permeability to induce contraction (HAGIWARA and KIDOKORO, 1971; HAGIWARA *et al.*, 1971).

According to FEINSTEIN (1966) and FEINSTEIN and PAIMRE (1969), local anesthetics nonspecifically inhibited the contraction of smooth muscle, *i.e.*, spontaneous rhythmic contractions, K^+ -contracture and Ach-contracture. And these inhibitory effects were thought to be due to the suppression of Ca^{++} permeation through the cell membrane and the intracellular membrane system. BURNSTOCK *et al.* (1966), however, reported that procaine caused a rise in tone of the taenia coli of the guinea pig. In the present experiment also, procaine induced a marked contracture in the bullfrog urinary bladder smooth muscle. Since this contracture was not inhibited by TTX at all, it is unlikely that the contracture is triggered by depolarization due to rapid Na^+ influx. On the other hand, the procaine-induced contracture was

perfectly inhibited by Mn^{++} , which has been related to E-C coupling of smooth muscle (NONOMURA *et al.*, 1966; BÜLBRING and TOMITA, 1969).

In smooth muscle, RCC has been observed even in the absence of caffeine (SAKAI and IIZUKA, 1972; MAGARIBUCHI *et al.*, 1973; KURIHARA *et al.*, 1973). In a guinea pig urinary bladder smooth muscle, strong contraction was triggered by rapid cooling. In this case, the depolarization of the membrane potential was observed, but the membrane resistance was increased more than twice. RCC was also observed in K^+ -depolarized muscle. Therefore, the RCC in the guinea pig urinary bladder was considered to be induced by Ca^{++} mobilization from the cell membrane and the intracellular membrane system (KURIHARA *et al.*, 1973). No detailed electrophysiological studies have been made on the RCC of the bullfrog urinary bladder, although here, too, RCC considered to be due to the Ca^{++} mobilization induced by rapid cooling.

According to SAKAI (1963) and SAKAI and IIZUKA (1972), procaine inhibited the caffeine-RCC of frog skeletal muscle and toad urinary bladder smooth muscle. On the other hand, WEBER and HERZ (1968) reported that procaine inhibited the release of Ca^{++} induced by caffeine. From these findings it is thought that procaine inhibits the caffeine-RCC because it inhibits the release of Ca^{++} from the sarcoplasmic reticulum induced by caffeine and rapid cooling. From the present experiment, however, in the bullfrog urinary bladder smooth muscle procaine might be considered to facilitate the Ca^{++} mobilization from its binding sites but not to increase the Ca^{++} influx from extracellular space primarily.

On the other hand, caffeine is said to inhibit the tonic response of K^+ -contracture in the rat intestine, the guinea pig taenia coli, and the toad urinary bladder smooth muscle (SUNANO, 1966; ITO and KURIYAMA, 1971; SAKAI and IIZUKA, 1972). ITO and KURIYAMA (1971) suggested that caffeine might accelerate the release of all bound Ca^{++} in the early stage so rapidly at 36°C, that the tissue would then relax without generation of the tonic response. The present experiment showed that in the bullfrog urinary bladder smooth muscle, procaine transiently reduced the tonic response of K^+ -contracture, but it potentiated the RCC. The mechanism of this relaxation was assumed to be the same as above, that is, Ca^{++} mobilized by procaine failed to induce contraction at room temperature. There was possibility that this relaxation was initiated by the inhibition of Ca^{++} permeation through the cell membrane as suggested by FEINSTEIN (1966) and FEINSTEIN and PAIMRE (1969). However, when the Ca^{++} mobilization was facilitated under the action of procaine, it was further accelerated by rapid cooling, and thus mobilized Ca^{++} might induce contraction. The effect of procaine on the bullfrog urinary bladder smooth muscle was evidently different from that reported by FEINSTEIN (1966) and FEINSTEIN and PAIMRE (1969), and SAKAI and IIZUKA (1972). At present the cause of this difference of opinion has not been clarified.

It has recently been reported that a charged form of procaine in a solution with low pH displaces bound Ca^{++} in the cell membrane of skeletal muscle fiber

and inhibits Ca^{++} release from the sarcoplasmic reticulum, but an uncharged form of procaine in a solution of high pH exerts a Ca^{++} releasing effect on the sarcoplasmic reticulum (BIANCHI and BOLTON, 1967; FEINSTEIN and PAIMRE, 1969; BONDANI and KARLER, 1970; THORPE and SEEMAN, 1971; MADEIRA and CARVALHO, 1972). ISAACSON *et al.* (1970) observed that procaine caused sizable contracture of skeletal muscle fibers of rats, and that the procaine-induced contracture of slow muscle fiber was stronger than that of fast muscle fiber. In the present experiment, also, in the bullfrog urinary bladder smooth muscle the procaine-induced contracture was enhanced in a high pH Ringer's solution. These findings suggest that procaine in a low pH solution mainly displaces bound Ca^{++} in the cell membrane, and that procaine in a high pH solution releases bound Ca^{++} in the intracellular membrane system of the tissue. It is thought that this Ca^{++} mobilization potentiates the RCC.

On the other hand, the present experiment showed that Mn^{++} inhibited the spontaneous rhythmic contractions, the procaine-induced contracture, and the RCC of the bullfrog urinary bladder smooth muscle, as well as the RCC-potentiating action of procaine. In smooth muscle, Mn^{++} has an inhibitory effect on E-C coupling, and can replace Ca^{++} for stabilization of the membrane but cannot replace Ca^{++} for the spike (NONOMURA *et al.*, 1966; BÜLBRING and TOMITA, 1969). Since Mn^{++} also inhibited the spontaneous rhythmic contractions and the RCC of the bullfrog urinary bladder smooth muscle, it is assumed that it inhibits the Ca^{++} influx from extracellular space and the Ca^{++} mobilization by acting on its binding sites. But it is thought that Mn^{++} partly replaces bound Ca^{++} , because it elevates the resting tension gradually throughout the experiment. IMAI and TAKEDA (1967) also reported that Mn^{++} augmented the tonic response of K^{+} -contracture of guinea pig taenia coli. All these findings indicate that Mn^{++} replaces bound Ca^{++} and inhibits the Ca^{++} mobilization caused by procaine and rapid cooling in the bullfrog urinary bladder smooth muscle. The mechanism of relaxation caused by rapid cooling in potassium Ringer's solution containing Mn^{++} has not been clarified. If it is postulated that Mn^{++} is released from its binding sites by rapid cooling, as in the case of Ca^{++} , and that Mn^{++} released by rapid cooling cannot induce contraction, rapid cooling treatment induces relaxation in potassium Ringer's solution containing Mn^{++} .

The mechanism of the action of procaine is not yet clear, but the present experiment suggests that procaine may mobilize bound Ca^{++} by acting on its binding sites in the cell membrane and the intracellular membrane system. On the other hand, Mn^{++} may replace bound Ca^{++} and inhibit the Ca^{++} mobilization caused by procaine and rapid cooling.

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