DESENSITIZATION OF SMOOTH MUSCLE CELLS IN THE GUINEA PIG TAENIA COLI TO PROLONGED APPLICATION OF CARBACHOL

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Summary To investigate the mechanism of desensitization to carbachol (Carb) in smooth muscle, the effects of prolonged applications of Carb on the electrical and mechanical properties of the guinea pig taenia coli were examined by either the microelectrode method or the double sucrose gap method.

1. Prolonged application of Carb ($10^{-5} \text{g/ml; } 5.5 \times 10^{-5} \text{M}$) initially depolarized the membrane, reduced the membrane resistance and evoked contracture. These responses did not, however, continue but a desensitization to Carb then developed, i.e. the membrane was gradually repolarized, membrane resistance increased, and the muscle relaxed in spite of the presence of Carb.

2. During the repolarization phase in the presence of Carb ($10^{-5} \text{g/ml}$), repeated current pulses transiently produced larger electrotonic potentials than those expected from the electrical displacement of the membrane potential. This transient increase of the membrane resistance was not directly related to the desensitization and it might be accounted for by a reduction of K permeability in the extrareceptor region at the depolarized condition.

3. Desensitization was accelerated in modified Krebs solutions with reduced Na or excess Ca, but was suppressed in solutions either with reduced Ca, or with reduced Cl or with excess K.

4. Procaine ($27 \times 10^{-5} \text{g/ml; } 1 \text{mM}$) suppressed both the depolarization and the desensitization induced by Carb. Hypertonic Krebs solution also suppressed the action of Carb on the taenia coli.

5. The contrast between the actions of Carb on the taenia coli and frog motor endplate has been discussed in terms of the ionic mechanisms involved.

It is well known that acetylcholine (Ach) and carbachol (carbamylcholine;
Carb) depolarize the membrane and potentiate the contraction of the guinea pig taenia coli. These phenomena are thought to be due to an increase of the membrane conductance to Na, K, and Ca and possibly to other cations, and if it increases Cl conductance, this increase is small compared to the increase in cation conductance. Ion flux studies in both normal and in depolarized muscles have indicated that Ach and Carb may increase permeability to K, Cl, Br, Na, and Ca (Bülbring, 1955; Burnstock, 1958; Bülbring and Kuriyama, 1963; Bennett, 1966; Burgen and Spero, 1968; Jenkinson and Morton, 1967; Kuriyama, 1970). Ach and Carb are also known to release the sequestered Ca from the internal membrane in the smooth muscles. For example, after the membrane is completely depolarized by excess K, Ach and Carb are still capable of evoking an additional contracture without any marked change of the membrane potential. Ach and Carb also produce a contracture when Ca influx is absent or suppressed (Edman and Schild, 1963; Schild, 1964; Imai and Takeda, 1967; Magaribuchi et al., 1973).

In many smooth muscles, prolonged treatment with either Ach or Carb initially induces contracture, but the muscles subsequently relax in spite of the continued presence of the drug. This phenomenon has been called desensitization or fade. The desensitizing effect on smooth muscle can also be induced by repeated applications of Carb or Ach separated by short intervals of time. For example, such application of the drug gradually reduced the peak amplitude of successive mechanical responses and the reduction of the amplitude was a function of the length of intervals between periods of drug applications (Paton, 1964; Waud, 1968; Rang and Ritter, 1970).

The present experiments were carried out to investigate the ionic mechanism underlying the desensitization phenomenon produced by prolonged application of Carb in the guinea pig taenia coli. Changes of membrane potential and resistance were measured in various ionic environments either by the micro-electrode method or the double sucrose gap method. The mechanical response was measured with a strain gauge.

METHODS

Guinea pigs weighing 250–350 g were stunned and bled. The taenia coli (20–25 mm in length) was excised from the caecum, and connective tissue was carefully removed.

The double sucrose gap method for recording electrical and mechanical activities was the same as that described by Kuriyama and Tomita (1970) and Magaribuchi et al. (1973). Changes in isometric tension were recorded with a strain gauge attached to one end of the tissue strip, and the stimulating electrodes were applied at the other. The applied stimulating current was usually $1-10 \times 10^{-7}$ amp.
Intracellular potentials from single smooth muscle cells were recorded with glass microelectrodes (Bülbring and Kuriyama, 1963). Each of the stimulating electrodes was a piece of chloride silver plate of 5×10 mm in size with a hole in the centre. They were placed in the experimental chamber about 10 mm apart, and one end of the preparation was passed through the holes. A stimulating current was applied between them, and the current intensity was monitored by two Ag–AgCl needle electrodes placed between the two plates (Tomita, 1970). In the text the current intensity is expressed as V/cm.

The following modified Krebs solution was used (mM): Na+, 137.4; K+, 5.9; Mg++, 1.2; Ca++, 2.5; Cl–, 134.0; HCO3–, 15.5; H2PO4, 1.2 and glucose, 11.5; equilibrated with 97% O2 and 3% CO2. The Na-deficient solution was prepared by replacement of NaCl with Tris-Cl or sucrose, so that 15.5 mM Na still remained in the solution. The pH of the solution was kept at 7.2–7.3 by Tris buffer. The K-deficient solution was prepared by replacement of KCl with NaCl and a part of KH2PO4 with NaH2PO4 isotonicly, so that the total K concentration was one-tenth the normal concentration. The excess-K solution was prepared by substituting isotonic KCl for NaCl. The Ca-deficient solutions were prepared to give concentrations of external Ca of 0.25, 0.5, and 1.25 mM. The excess-Ca Krebs (12.5 mM) was prepared by addition of solid CaCl2 to the solution, and to prevent the precipitation of Ca, NaHCO3 was reduced to a half by substitution of NaCl, and the pH was adjusted by Tris buffer. The Cl-deficient solution was prepared by replacements of Cl from NaCl and KCl with either C6H5SO3 or Br but still 7.4 mM Cl was contained as CaCl2 and MgCl2.

Drugs used: carbachol HCl (Carbamylcholine chloride; Sigma Chem. Co., Ltd.); Procaine HCl (Procaine hydrochloride; Daiichi Pharm. Co., Ltd.). The concentrations of Carb were varied between 10^-8 g/ml (5.5×10^-8 M) and 10^-5 g/ml (5.5×10^-5 M), and mainly 10^-5 g/ml was used.

RESULTS

Effects of prolonged application of Carb on the electrical and mechanical activity of the smooth muscle

With a concentration of 10^-8 g/ml, Carb depolarized the membrane (6 mV, n=5, double sucrose gap method: Dsg) and reduced the membrane resistance (0.68 times the control value, n=5, Dsg). When the concentration of Carb was increased to 10^-5 g/ml, the membrane was depolarized further (4.1 mV, n=5, Dsg) and the membrane resistance was reduced to approximately 0.02 times the control value (n=5, Dsg). The membrane resistance during the peak depolarization could not be accurately measured, however, because of the marked reduction of the electrotonic potential. Moreover, when the membrane resistance is reduced to one one-hundredth, the length constant of the tissue must be reduced to one-tenth on the assumption that the diameter and the
Fig. 1. Effects of carbachol (10^{-8}-10^{-5} g/ml) on relative membrane resistance, membrane potential and tension development in the guinea-pig taenia coli. Carbachol was applied for 3 min. a) Relative changes of the membrane resistance. b) Changes of the membrane potential. c) Developments of the mechanical responses. From a) to c), plotted responses against the time. Responses of the tissue were recorded by the double sucrose gap method.

longitudinal resistance of the tissue remain the same. In the taenia coli, the length constant is 1.5 mm (Tomita, 1970). The width in the central region of the gap apparatus was 0.5 mm in the present experiment. Therefore reduction of the length constant might reduce the observed amplitude of the electrotonic potential, which would no longer simply be proportional to the membrane resistance.

During the depolarization phase evoked by Carb, bursts of spike discharges
were generated. But when the membrane depolarization exceeded 14 mV (n=20, microelectrode; M.E.), the bursts ceased, probably as a result of depolarization block.

Figure 1 shows the effects of Carb (10^{-8}-10^{-5} g/ml) on the membrane resistance (a), membrane potential (b), and mechanical responses (c) of the taenia coli measured with the double sucrose gap method. The dotted lines in the figure indicate the periods of spike generation. At concentrations of 10^{-8} and 10^{-7} g/ml, Carb depolarized the membrane and generated spikes during depolarization, thus evoking tension development. At higher concentrations (10^{-6} and 10^{-5} g/ml), the reduction of membrane resistance, depolarization and amplitude of the mechanical response (Carb contracture) were greater than those observed at 10^{-7} and 10^{-8} g/ml. However, these responses of the tissue gradually decayed, in spite of the continued presence of Carb. When the tissue was rinsed with Krebs solution, the membrane was transiently repolarized to a level more negative than the control level, especially after exposure to concentrations of 10^{-7} and 10^{-6} g/ml Carb. This phenomenon is presumably due to an activation of the electrogenic Na pump (BOLTON, 1971).

As described previously, Carb (10^{-5} g/ml) initially produced marked depolarization (−7 mV, n=20, microelectrode; M.E., 41 mV, Dsg), reduced membrane resistance (0.02 times the control value, Dsg) and evoked contracture. During the repolarization phase, the amplitude of the electrotonic potential evoked by an inward current pulse was gradually increased. When the mem-
brane was repolarized to a certain level, however, the membrane resistance was again reduced, and in some preparations it attained a value even smaller than that measured at peak depolarization. In parallel with the repolarization of the membrane, the muscle relaxed.

As shown in Fig. 2, successively applied inward current pulse did not influence the electrical and mechanical responses evoked by Carb, the time course of the membrane and mechanical responses evoked by Carb being nearly identical irrespective of the presence or absence of the weak inward current pulses. Evidence that these changes in the tissue to prolonged application of Carb were not artifacts due to the use of the double sucrose gap method is provided by the fact that similar phenomena were also observed with the microelectrode method (not shown).

![Fig. 3. Effects of carbachol (10^-5 g/ml) on the electrical activity and current-voltage relations of the membrane in the taenia coli measured by the microelectrode method. The responses of the tissue were measured in Cl-deficient (C₆H₅SO₃) Krebs solution. Still 7.4 mM Cl remained as CaCl₂ and MgCl₂. b, c, and d in the figure show the actual records of the membrane potential changes produced by various intensities of current pulse in the presence of carbachol. Parenthesis in the upper figure (b, c, and d) correspond to parenthesis in the I-V relation curve (b, c, and d). a) Depolarization phase of the membrane produced by carbachol. e) Current-voltage relationship observed after the tissue was repolarized in the presence of carbachol. O, Onset of application of carbachol.](image-url)
To measure the change in the membrane resistance during the repolarization phase, the effects of Carb (10^{-5} g/ml) on the membrane activity of the smooth muscle and on the current-voltage relationship were measured at 200\mu with the stimulating electrode (Fig. 3). The obtained current-voltage curves are only approximations, because the membrane potential was not always constant and spikes were superimposed. Further inaccuracy was probably also introduced by a reduction of the length constant. However, the V–I relations measured at various phases of repolarization were not the same in shape and the membrane resistance showed higher value than that of less depolarized level over a certain potential range.

Relaxation of the Carb-induced contracture in the continued presence of Carb was not due to muscle fatigue, because when an outward current pulse was applied to the tissue after the tissue was relaxed, twitch tension was evoked which was even larger than that evoked in normal Krebs solution.

Effects of various conditions on the actions of Carb to the smooth muscles

**Calcium.** Low Ca (0.25 mM) reduced the size of the depolarization, delayed its appearance, and prolonged the period of the contracture, which were produced by application of Carb. Excess Ca (7.5 mM) accelerated the appearance of the desensitization, increased the size of depolarization and contracture evoked by Carb but the relaxation started much earlier than in Krebs solution. In low Ca but not in excess Ca Krebs solution, transient increase of the membrane resistance during the repolarization of the membrane was observed.

Figure 4 summarized the effects of Carb (10^{-5} g/ml) on the membrane potential and mechanical responses in solutions with various concentrations of Ca (0.25–10 mM). In (a), amplitudes and durations of the depolarization induced by Carb and in (b) amplitudes and durations of the Carb contracture in the various concentrations of Ca were illustrated. As shown in the figure, in low Ca (<2.5 mM), less depolarization of the membrane with prolongation of the repolarization phase was observed and the sequences were reversed in excess Ca (>2.5 mM). The mechanical responses were followed by changes in the membrane potential.

**Strontium.** Sr accelerates spike generation and is substituted for Ca in the spike generation (SAKAMOTO, 1970). On pretreatment with Sr, Carb (10^{-5} g/ml) still depolarized the membrane and induced contracture, but the amplitudes of the depolarization and contracture became small and the repolarization phase became longer than in Krebs solution (half repolarization time was 2.8 min and 1.5 min, respectively; n=5, Dsg). The spike appeared during exposure to the drug because of a depolarization of the membrane (21 mV, n=3, Dsg).

**Potassium.** At low concentrations of K (0.59 mM), the membrane was slightly hyperpolarized (5 mV, n=20; M.E.). Carb (10^{-5} g/ml) depolarized the membrane (−11 mV, n=5; M.E.), reduced the membrane resistance (0.04 times the
Fig. 4. Effect of carbachol ($10^{-5}$ g/ml) in Krebs solution with various concentrations of Ca (0.25–10 mM). Carbachol was applied for 3 min. a) Effects of Ca concentration on size and duration of the membrane depolarization evoked by carbachol. b) Effects of Ca concentration on amplitude (relative amplitude; control=1) and duration of the mechanical responses evoked by carbachol. Ion a and b, dots show mean values of four experiments and vertical lines show ± S.D.

control value, $n=5$, M.E.) and produced transient increase of the membrane resistance during the repolarization phase. These responses of the tissue to Carb in low K Krebs solution were nearly the same as those observed in Krebs solution.

In contrast with the effects of Carb in Krebs solution, Carb contracture was maintained in Krebs solution with excess K. Figure 5 shows the effects of Carb ($10^{-5}$ g/ml) in standard Krebs and 118 mM K Krebs solutions. In Krebs solution Carb-contracture relaxed within 2 min (a). Excess K Krebs solution produced sustained depolarization of the membrane and a phasic contraction was followed by a sustained contraction (b). Carb barely changed the membrane potential further, but it induced an additional contracture superimposed on the K-induced contracture. The contracture lasted as long as the Carb existed in the outside medium with very little decay.
CARB. DESSENSITIZATION IN SMOOTH MUSCLE

Fig. 5. Effects of carbachol (10^{-5} g/ml) on the taenia coli in Krebs solution and in excess K Krebs solution (118 mM K). Upper trace; tension. Lower trace; membrane potential. a) 10^{-5} g/ml carbachol. b) 10^{-5} g/ml carbachol in 118 mM K-Krebs solution. Inward current pulse, 2 \times 10^{-7} A; Outward current pulse, 5 \times 10^{-7} A. The double sucrose gap method was used.

Chloride. Substitution of Cl with Br in Krebs solution hyperpolarized the membrane (4 mV, n=20, M.E.), reduced the membrane resistance (0.91 times the control, n=3, M.E.) and increased the spike frequency (1.28 times the control, n=3). In the presence of Br, desensitization to Carb was suppressed and repolarization became slow. Appearance of the transient increase of the membrane resistance was also delayed.

In the solution containing C_6H_5SO_3, Carb depolarized the membrane. During the repolarization phase, the membrane potential fluctuated markedly between two potential levels. Figure 6 shows the effects of Carb (10^{-7}, 10^{-6}, and 10^{-5} g/ml) on the electrical and mechanical activities of the taenia coli in Cl-deficient (C_6H_5SO_3) Krebs solution. During the repolarization phase in treatment with high concentration of Carb, marked changes in membrane resistance were observed, i.e. at the lower membrane potential level, inward current pulses produced larger electrotonic potentials than those produced at the higher membrane potential level. These fluctuations of the membrane potential accompanying the paradoxical changes of the membrane resistance were presumably due to an enhancement of a mechanism which is responsible for the transient increase of the membrane resistance produced by Carb in standard Krebs solution (see Figs. 1a and 3). The same mechanism might also be responsible for the delayed appearance of the desensitization (see DISCUSSION). In fact, such fluctuations of the membrane potential with the changes of the membrane resistance were also observed in the absence of Carb when the preparation was immersed in Cl-deficient (C_6H_5SO_3) excess K Krebs solution over a certain membrane potential range (from -31 to -24 mV; n=5). As shown by KURIYAMA (1963), when
Fig. 6. Effects of carbachol (10⁻⁷, 10⁻⁶, and 10⁻⁵ g/ml) on the electrical and mechanical properties of the smooth muscle in Cl-deficient (C₆H₅SO₃) Krebs solution. NaCl and KCl in Krebs solution were replaced with NaC₆H₅SO₃ and KC₆H₅SO₃. Inward current pulses (2×10⁻⁷ A) were successively applied. Electrical and mechanical activities were determined by the double sucrose gap method.

Fig. 7. Effects of 29.5 mM K- and 59 mM K-Cl deficient (C₆H₅SO₃) Krebs solution on electrical and mechanical activities of the taenia coli. Double sucrose gap method. a) With successive pulses. b) Without pulse during the application of 29.5 mM K-Krebs solution. c) Without pulse during the application of 59 mM K-Krebs solution. Dots in b and c indicate the time of applications of electrical stimulation (inward and outward current pulses; 3×10⁻⁷ A and 6×10⁻⁷ A, respectively).
Cl in the excess K Krebs solution was replaced with C$_6$H$_5$SO$_3$, the membrane was further depolarized than that in the presence of Cl. Figure 7 shows such an experiment using 29.5 mM KC$_6$H$_5$SO$_3$- and 59 mM KC$_6$H$_5$SO$_3$-Krebs solution with the double sucrose gap method. In (a), inward current pulses were successively applied to the tissue. During the application of 29.5 mM KC$_6$H$_5$SO$_3$-Krebs solution, membrane resistance was higher at the more depolarized level than that at the less depolarized level. With or without application of the pulses, the responses of the muscle to 29.5 mM KC$_6$H$_5$SO$_3$-Krebs solution were the same (b). An increase of K concentration to 59 mM further depolarized the membrane and no transition of the membrane potential was observed (c). When inward current pulse were applied during the sustained depolarization phase, more reduction of the membrane resistance was observed than that measured in 29.5 mM K-Krebs.

**Fig. 8.** Effects of carbachol (10$^{-7}$-10$^{-5}$ g/ml) on the electrical and mechanical activities of the taenia coli in Na-deficient Krebs solution. NaCl was substituted with sucrose. Inward and outward current pulses were applied to the tissue (2×10$^{-7}$ and 6×10$^{-7}$ A, respectively). a) 10$^{-7}$ g/ml carbachol. b) 10$^{-6}$ g/ml carbachol. c) 10$^{-5}$ g/ml carbachol. Responses of the tissue were recorded by the microelectrode method.

**Sodium.** After replacement of Na with either Tris or sucrose, the membrane was hyperpolarized (6 mV, n=35, M.E.) in both solutions, but the membrane resistance was either reduced (0.68 times the control value in Tris Krebs solution, n=3, Dsg) or increased (1.41 times the control value in sucrose Krebs solution, n=3, Dsg). In a concentration of 10$^{-5}$ g/ml, Carb-induced depolarization was small and the repolarization appeared earlier and faster than in Krebs solution. No transient increase of the membrane resistance appeared during the repolarization phase. Figure 8 shows an example of the effects of Carb (10$^{-7}$-10$^{-5}$ g/ml) on the electrical and mechanical activities of the smooth muscle in Na-deficient
(sucrose) Krebs solution observed with the double sucrose gap method. In this solution, 15.5 mM Na remained as NaHCO₃. Changes of the membrane potential and of the membrane resistance were smaller than those observed in the Na-free Krebs solution (3 mV, n=5, Dsg and 1.21 times the control value, respectively). However, desensitization to Carb was still accelerated, i.e. repolarization appeared faster and the membrane resistance was gradually increased to the control value without any appearance of transient increase of membrane resistance.

**Procaine.** Procaine (27×10⁻⁵ g/ml; 1 mM) slightly depolarized the membrane (4 mV, n=4, Dsg; 5 mV, n=20, M.E.), increased the membrane resistance (1.21 times the control value, n=4, M.E.) and accelerated the spike generations. Figure 9 shows the effects of procaine (27×10⁻⁵ g/ml) on the electrical activity of the taenia coli, when inward current pulses were successively applied to measure changes of membrane resistance. Procaine depolarized the membrane and generated spike discharges which were only suppressed during applications of the inward current pulse. Figure 10 shows an example of the effects of Carb (10⁻⁵ g/ml) in the presence of procaine (27×10⁻⁵ g/ml). In this preparation, the effects of Carb on the muscle were completely suppressed. Not only in such an extreme example but in all the preparations (n=8) procaine reduced the effects of Carb, i.e. depolarization of the membrane, reduction of the membrane resistance and the amplitude of the mechanical response became small. This suppression of the effects of Carb by procaine was partly antagonized by treatment with excess Ca.

**Osmolarity.** In the motor endplate of frog skeletal muscle, it has been reported that hypertonic solution accelerated the Carb-induced desensitization (NUSTUK and PARSON, 1970), and the effects of Carb on the electrical and mechanical properties of the taenia coli have, therefore, also been studied in hypertonic

![Fig. 9. Effects of procaine (1 mM: 27×10⁻⁵ g/ml) on the electrical activity of the taenia coli. ⊗, Application of procaine. Intensity of inward current pulses, 4 V/cm. Responses of the membrane were recorded by the microelectrode method.](image-url)
CARB. DESENSITIZATION IN SMOOTH MUSCLE

Fig. 10. Effects of carbachol (10⁻⁵ g/ml) before, during and after application of procaine (1 mM; 27×10⁻⁵ g/ml). a) Before application of procain. b) During application of procaine. c) After rinse with Krebs solution. Inward and outward current pulses were applied to the tissue (3×10⁻⁷ A and 8×10⁻⁷ A, respectively). Tissue responses were determined by the double sucrose gap method.

solution. Hypertonic Krebs solution was prepared by the addition of solid sucrose (90 g) to 1 liter Krebs solution. In this solution the effects of Carb (10⁻⁵ g/ml) were markedly reduced. As a consequence the Carb-induced contracture was smaller in amplitude. No anomalous rectifying property of the membrane during the repolarization phase was observed.

DISCUSSION

In various smooth muscles, the application of high concentrations of stimulating agents produces contracture, but the muscles eventually relax even in the continued presence of the agents. This phenomenon has been called desensitization or fade (GADDUM, 1957; PATON, 1964; WAUD, 1968; RANG and RITTER, 1970).

In the motor endplate, the desensitization (KATZ and THESEFF, 1957) or inactivation (NASTUK and GISSEN, 1966) has been studied by electrophysiological methods, especially the measuring of ionic conductance. However, in the smooth muscle, desensitization was deduced from the relaxation of the tissue in the pre-
sence of stimulating agents. Recently, Johnson and Marshall (1972) observed the effects of prolonged application of Carb on the rat myometrium by electrophysiological methods and their results indicated that membrane repolarization coincided with the decline in tension during desensitization, but that the time course of desensitization was not affected by Ca. On the other hand, Osa and Taga (1973) reported that depolarization by Carb was succeeded by repolarization in the continued presence of Carb in the mouse myometrium, and that excess Ca accelerated the appearance of desensitization.

In the present experiments, desensitization was designated from the repolarization of the membrane after depolarization, and the return of the membrane resistance after a fall, and these membrane changes coincided with relaxation of contracture. In the smooth muscles of taenia coli, it is difficult to classify the receptor and extra-receptor regions by morphological and physiological methods, and consequently application of the point-clamp method and iontophoretic release of a drug close to the receptors is difficult (Ginsborg, 1967). This means that the results obtained in the smooth muscle membrane might not be comparable with those observed in the motor endplate. However, the desensitization phenomena observed in the smooth muscle of the taenia coli and the frog motor endplate showed several similarities; 1) repolarization of the membrane was accompanied by an increase of membrane resistance, 2) increase of Ca concentration accelerated desensitization, 3) Sr was a very poor substitute for Ca in causing desensitization, 4) excess K suppressed desensitization (Manthey, 1970; Lambert and Parson, 1970; Nastuk and Parson, 1970). Differences observed between the behaviour of the two tissues were as follows; 1) in the motor endplate, desensitization was accelerated in hypertonic solution but not in the taenia coli. 2) during the repolarization phase in the presence of Carb, transient increase of the membrane resistance was observed in the taenia coli but not in the motor endplate. 3) Cl-deficient solution by substitution of Cl with a less permeable anion accelerated desensitization in the motor endplate, but suppressed it in taenia coli. 4) excess Ca accelerated the desensitization to Carb in both the tissues, but the repolarization was accompanied by a rapid increase of the membrane resistance in the motor endplate but not in taenia coli.

Procaine presumably suppressed the K conductance in taenia coli, thus depolarizing the membrane and evoking the spikes. These effects may be compared with the responses observed in the presence of tetraethylammonium, which suppress the K conductance of smooth muscles (Ito et al., 1970). When Carb was applied to the tissue after pretreatment with procaine, the reduced response was observed which might imply that procaine additionally suppressed the increase of the Na conductance.

In the present experiments, transient increase of the membrane resistance during the depolarization of the membrane appeared under two different conditions; (1) during desensitization to Carb, and (2) in Cl-deficient ($\text{C}_6\text{H}_5\text{SO}_3$) excess
K Krebs solution (29.5 mM). As described previously, desensitization and transient increase of the membrane resistance are probably independent of each other, and it may be inferred that the former is caused by changes of the receptor and the latter by changes of the extra-receptor membrane.

Transient increase of the membrane resistance during the repolarization phase was time- and voltage-dependent process (Fig. 3) and a termination of this phenomenon tended to repolarize the membrane further to the resting membrane potential level (Fig. 9a and c). The Carb-induced transient increase of the membrane resistance appeared in K-deficient, Cl-deficient and Ca-deficient solutions, including standard Krebs solution, but disappeared in excess-K, Na-free, and excess-Ca Krebs solutions. This mechanism might, therefore, be related to the reduction of the K permeability over a certain membrane potential range, since in the taenia coli, the Cl-deficient solution suppressed the K permeability but Na-free and excess-Ca Krebs solutions accelerated it (BRADING et al., 1969; KURIYAMA, 1970).

It is known that the K system in skeletal muscle and in Purkinje fibres in Krebs solution shows inward-going (anomalous) rectification, i.e. the K permeability, $P_k$, is increased by an inward electrochemical potential gradient and an outward electrochemical potential gradient has the effects of reducing $P_k$ (KATZ, 1949; ADRIAN and FREYGANG, 1962; NOBLE, 1965; NOBLE and TSIEN, 1969). This phenomenon is thought to be related to a function of the internal membrane system in skeletal muscle and to be related to holding the membrane potential at a plateau phase during the active state in cardiac muscle (ADRIAN and FREYGANG, 1962; NOBLE, 1965). Presumably a similar mechanism might take place to generate both the transient increase of the membrane resistance in smooth muscle and inward-going rectification of the membrane in cardiac and skeletal muscles over a certain membrane potential level.

Prolonged application of Carb might drastically modify intracellular ionic concentrations. As a consequence the equilibrium potentials for each of the ions contributing to the generation of the membrane potential would be changed. For generation of transient increase of the membrane resistance, increased internal Na and reduced internal K due to treatment with Carb might play a role. Such a change in the intracellular ionic composition of the smooth muscle is equivalent to the reduction of K conductance at low K equilibrium potential observed in the frog skeletal muscle.

In excess Ca Krebs and in Na-free solutions, desensitization to Carb was enhanced, and the increased concentration of Ca might suppress the reduction of K conductance of the plasma membrane, while the reduced Na in Krebs solution might reduce the depolarization and also partly increase K conductance (BRADING et al., 1969), thus suppressing the appearance of transient increase of the membrane resistance during the repolarization phase.

NASTUK and PARSON (1970) postulated that in the motor endplate receptor
inactivation produced by Carb is accelerated by a rise in intracellular Ca concentration which promotes the reaction of Ca with anionic sites on the inner surface of the post junctional membrane, thus suppressing the ionic permeability especially to Na and K ion, at the receptor. They also suggested that increased Ca concentration in the cell has an action on the plasma membrane in the striated muscle. Presumably a similar course of events may be followed in smooth muscle.

Nevertheless, from the present experiments it is too early to assume that the hypothesis concerning the desensitization to Carb, which was presented by either Katz and Thesleff (1957) or Nastuk and Parson (1970) on the motor endplate (i.e. parallel reaction theory, sequential reaction theory, cyclic reaction theory and two-site theory) can be applied directly to smooth muscle.

In a Na-deficient solution, Ca ions might be accumulated in the cell due to suppression of the Na-Ca exchange mechanism thus accelerating desensitization, while in a K-deficient solution and in a Cl-deficient solution, suppression of Ca accumulation to inside of the membrane might suppress generation of desensitization. Excess Ca might therefore accelerate desensitization (Sakamoto and Kuriyama, 1970; Tomita, 1970; Katase and Tomita, 1972). It may at least be postulated, however, that the changes of ionic permeabilities during desensitization are closely linked with the separate roles of Ca at receptor site and at the extra-receptor membrane.

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