Br. J. Pharmac. Chemother. (1966), **27**, 366–376.

EFFECT OF TETRODOTOXIN ON SMOOTH MUSCLE CELLS OF THE GUINEA-PIG TAENIA COLI

BY

H. KURIYAMA, T. OSA AND N. TOIDA

From the Department of Physiology, Faculty of Medicine, Kyushu University, Japan

(*Received March* 14, 1966)

In the smooth muscle of the guinea-pig taenia coli the maximum rate of rise of the action potential is low (5–10 v/sec). This has been attributed to a poorly developed sodium carrier system which may be largely inactivated (Holman, 1958; Bülbring & Kuriyama, 1963). The latter is, however, unlikely since Kuriyama & Tomita (1964a & b, 1965) observed that hyperpolarization of the membrane up to 90 mV increased the maximum rate of rise of the spike, but never reached the value observed in skeletal muscle. It is also known that the influence of sodium and calcium ions on spike generation in the smooth muscle membrane is different from that on other excitable cell membranes. Bülbring & Kuriyama (1963) found that the rate of rise, but not the amplitude of the spike, was related to the external sodium concentration. On the other hand, both the rate of rise and the amplitude of the spike were a function of the external calcium concentration. Furthermore, in the absence of sodium, the time during which spontaneous discharge continued, as well as the spike amplitude, were a function of the external calcium concentration. These results suggested the possibility that calcium, fixed in the membrane, might not only be important for spike generation by controlling the sodium-carrying channel, but could itself carry the current responsible for the spike.

The present experiments were carried out in an attempt to clarify the mechanism of spike generation in the smooth muscle cell of guinea-pig taenia coli with the aid of two pharmacological tools. The first, tetrodotoxin, has been shown to prevent the increase in sodium conductance during the active state, while the second, aconitine, is believed to prolong the increased sodium conductance after the action potential.

The effect of tetrodotoxin on excitable cell membranes has been studied by Narahashi and others (skeletal muscle: Narahashi, Deguchi, Urakawa & Ohkubo, 1960; giant nerve fibre, Narahashi, 1964; Narahashi, Moore & Scott, 1964; Nakamura, Nakajima & Grundfest, 1965; frog nerve fibre: Fleisher, Killos & Harrison, 1961; neuromuscular junction: Furukawa, Sasaoka & Hosoya, 1959). Narahashi, Moore & Scott (1964) measured sodium and potassium currents in the lobster giant axons treated with tetrodotoxin by means of the sucrose gap voltage clamp technique. They found that the increase in sodium conductance normally occurring upon depolarization was effectively suppressed when the action potential was blocked with tetrodotoxin, although the delayed increase in potassium conductance underwent no change. They put forward a working hypothesis on the action of tetrodotoxin, i.e., that the tetrodotoxin molecule binds with the membrane components in such a way that it prevents membrane calcium from being displaced by depolarization.

Aconitine is known to produce "fibrillation" and "flutter" of cardiac and skeletal muscle (Scherf, 1947; Scherf, Schaeffer & Blumenfeld, 1953; Matsuda, Hoshi & Kameyama, 1959; Tamai, Yanaga & Goto, 1961; Goto, Tamai & Yanaga, 1963). In nerve, the effect of aconitine is similar to that of veratrine, prolonging the negative after-potential following the spike (Graham & Gasser, 1931). The effect of aconitine on the membrane of the giant nerve fibre has been studied by Herzog, Feibel & Bryant (1964), who attributed the effect to an increase of Na and K or Cl conductances.

The results obtained in the work to be described have led to the general conclusion that the spike generation mechanism in smooth muscle of the guinea-pig taenia is qualitatively different from other excitable membranes, i.e., those of ventricular cardiac muscle and diaphragm muscle of the same species.

Some of the results have been communicated to the Physiological Society in Japan and the twenty-third International Congress of Physiological Sciences (Toida & Osa, 1965).

T. Nonomura (1965, personal communication) has also observed the effect of tetrodotoxin on the smooth muscle cell activity using the sucrose gap method, and his results are in complete agreement with our observations.

METHODS

The smooth muscle mainly used for these experiments was the longitudinal muscle of the taenia coli of guinea-pig. Guinea-pigs weighing 400–450 g were stunned and bled. The method of mounting a small length of taenia coli has been described previously by Bülbring (1957). Lengths from 4 to 6 mm were mounted on a perspex ring isometrically in an organ bath of 2 ml. capacity, made of perspex, through which solution flowed continuously at the rate of 2–3 ml./min, at a temperature of 35–36° C. In order to make a comparison between the responses of this smooth muscle to drugs and those of other excitable tissues, cardiac muscle and diaphragm of guinea-pig were used. A strip of the diaphragm (width 3 mm and length 10–15 mm) was excised and fixed at both ends of the tendon with cotton thread. The pacemaker, atrial and ventricular muscle of heart were used as the representatives of the cardiac muscle. The diaphragm and cardiac muscles were mounted in the same organ bath as that used for the taenia coli. The membrane potential and the spikes were measured, using the floating method (Woodbury and Brady, 1956), with intracellular electrodes with a resistance between 50–70 MΩ. The upper surface of the specimens was kept about 1 mm below the surface of the bathing solution. Intracellular polarizing current was applied to the cell, using the Wheatstone bridge method which has been described by Kuriyama & Tomita (1965). In order to supply a constant current into the cell, the resistance of one bridge arm in series with the microelectrode was fixed very much higher (1,000 MΩ) than that of the microelectrode. The electrodes used for extracellular polarization consisted of two silver-silver-chloride rings embedded 2 mm apart in insulating Araldite.

The modified Krebs solution used in all experiments contained (mM); Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, $H_2PO_4^-$ 1.2, HCO_3^- 15.5, glucose 11.5; and was oxygenated with 97% O_2+3% CO_2. 1 mg crystalline tetrodotoxin (Sankyo Co.) of which the LD50 for the mouse is 10^{-5} g per Kg, was dissolved in 10 ml. distilled water and kept at 4° C. Fresh stock solution was prepared at least every week. Aconitine was dissolved in ethanol and then diluted with the modified solution to a suitable concentration. Individual concentrations are described in the results.

RESULTS

Effects of tetrodotoxin on smooth muscle

The membrane potential (45–58 mV, n = 120), the spike amplitude (40–65 mV, n = 120), the maximum rates of rise and fall of the spike (4.5–10.5 v/sec and 4.2–9.8 v/sec, respectively, n = 30) were measured at 35–37° C. These observations confirmed previous

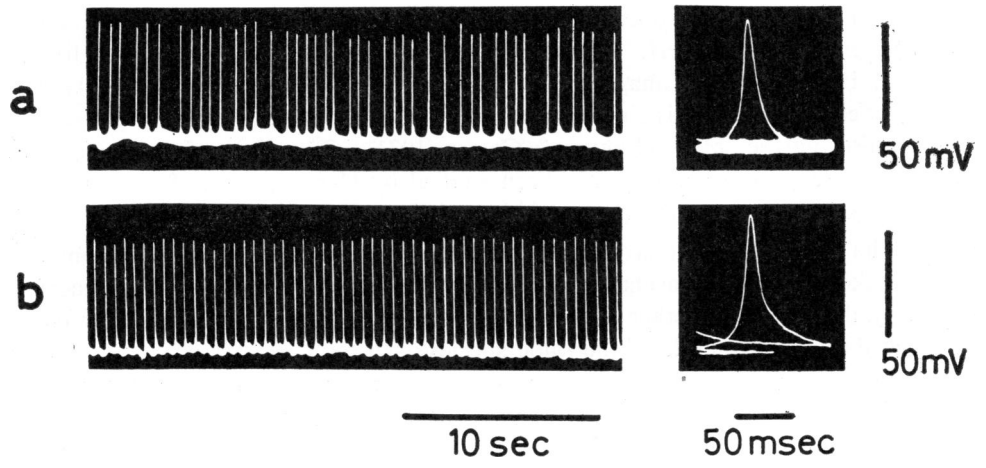

Fig. 1. Effect of tetrodotoxin (5×10^{-7} g/ml.) on the spontaneous discharges of the smooth muscle cells of guinea-pig taenia coli. Tetrodotoxin often increased the spike frequency. (a) Control. (b) Tetrodotoxin after 20 min.

reports (Holman, 1958; Bülbring & Kuriyama, 1963). $5–10 \times 10^{-7}$ g/ml. tetrodotoxin were used to observe the results of poisoning. These concentrations were about 100 times higher than those which blocked the spike generation in the skeletal muscle, giant nerve fibre and myelinated nerve fibre (cf. Narahashi, Moore & Scott, 1964), but the spike was not blocked in the smooth muscle. No effect was observed on the maximum rates of rise and fall of the spike. However, sometimes, tetrodotoxin increased the frequency of the spontaneous spike discharge. This is shown in Fig. 1. Tetrodotoxin (5×10^{-7} g/ml.) in this specimen, increased the spike frequency to 1.5–2.5 times that of control. The membrane was slightly depolarized because of the increased spike frequency. The active tension development decreased transiently on exposure to tetrodotoxin and then increased again. The relation between this transient change of tension development and the change of spike frequency was not studied precisely, but this phenomenon might be related to the transient irregularity of the spike frequency and the spike amplitude. The membrane activities of the taenia coli in presence of tetrodotoxin resembled those observed in the sodium-free solution. However, tetrodotoxin never hyperpolarized the membrane, and membrane activity continued for many hours in the presence of tetrodotoxin.

Effects of tetrodotoxin on cardiac and skeletal muscles
Cardiac muscle

Fig. 2 shows the effect of tetrodotoxin on a pacemaker and on a ventricular muscle cell of the guinea-pig heart. In the pacemaker cell, tetrodotoxin (5×10^{-7} g/ml.) did not block the spontaneous beat. In some preparations, however, the spike generation was blocked but, after extracellularly applied electrical stimulation, it reappeared with a slightly higher frequency than before. When the concentration of tetrodotoxin exceeded the above value, a spike could not be evoked by any method in the pacemaker cell. In contrast to the pacemaker, the spike activity of the ventricular muscle was completely blocked

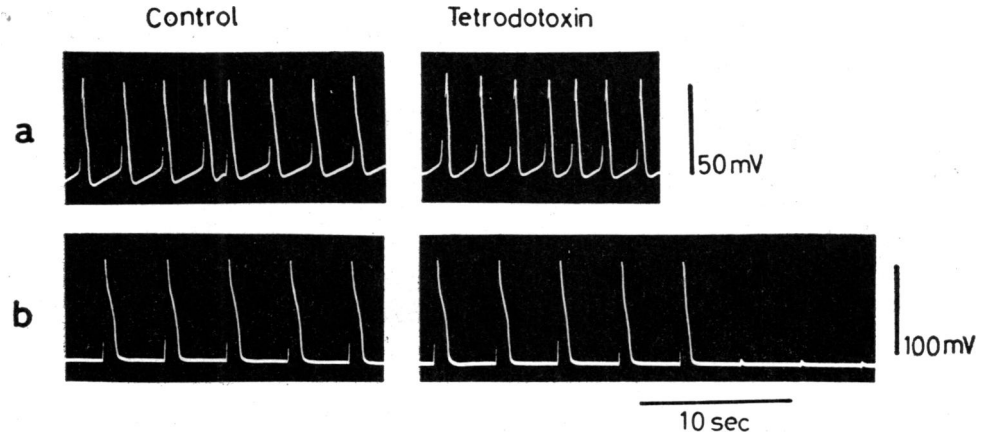

Fig. 2. Effect of tetrodotoxin (5×10^{-7} g/ml.) on the membrane activities of the pacemaker cell (a) and ventricular muscle cell (b) of guinea-pig heart. On the ventricular muscle, tetrodotoxin blocked the spike generation triggered by electrical stimulation (0.4 c/sec 5 msec) without any change of the membrane potential.

by tetrodotoxin 5×10^{-8} g/ml. and electrical stimulation failed to evoke a spike. In some experiments, the action of tetrodotoxin on the spike initially appeared on the plateau phase reducing its duration without influencing the spike amplitude. Then tetrodotoxin blocked the spike generation without preceding reduction of the spike amplitude and without change of the resting potential.

When 5×10^{-8} g/ml. tetrodotoxin was administered to the whole excised heart, superfused with modified Krebs solution on a cotton-wool support and observed under the microscope, only the beats of the pacemaker and the auriculoventricular node could be seen while the major portion of the heart was quiescent. This observation might indicate that the cells which have automaticity like the smooth muscle were resistant to tetrodotoxin.

Skeletal muscle

Tetrodotoxin ($5–10 \times 10^{-8}$ g/ml.) blocked the spike in the guinea-pig diaphragm muscle. Fig. 3 shows the effect of tetrodotoxin (10^{-7} g/ml.) on the action potential elicited by electrical stimulation. In this concentration the spike was promptly blocked without any change of the membrane potential.

When the diaphragm of the guinea-pig was immersed in the solution containing 1/5 of the normal Ca^{++} and K^{+} concentration, the membrane was slightly depolarized from 76–94 mV to 71–86 mV ($n=50$) and became unstable. The spontaneously discharged spike was preceded by a prepotential of pacemaker type or followed a slow potential change similar to that observed in smooth muscle. In this solution the membrane became very sensitive to mechanical stimulation such as the insertion or extraction of the microelectrode or the contraction of neighbouring cells, and stimulation triggered a train of discharges from this cell membrane. The shape and rhythm of the spike generated in this solution resembled those during spontaneous activity of smooth muscle (Bülbring,

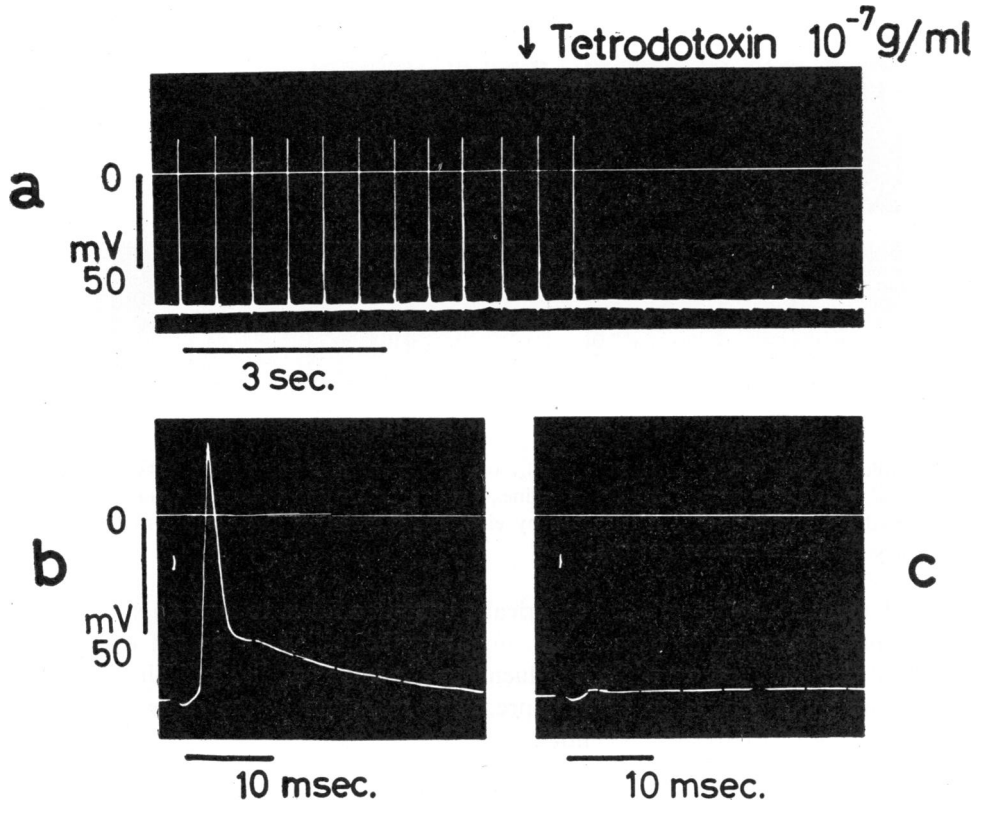

Fig. 3. Effect of tetrodotoxin (10^{-7} g/ml.) on the action potential of the skeletal muscle cell of the guinea-pig diaphragm. (b) and (c) are taken at faster speed than (a). (b) Control. (c) After treatment with tetrodotoxin (10^{-7} g/ml.).

Holman & Lüllman, 1956). Tetrodotoxin (10^{-7} g/ml.) completely blocked this spontaneous spike discharge in calcium deficient solution as well as the mechanically induced spike discharge.

Effect of tetrodotoxin on the membrane activity of smooth muscle during application of hyperpolarizing current and on the spikes triggered by intracellular stimulation

Intracellularly applied inward (hyperpolarizing) current enhanced the spike amplitude and the maximum rate of rise of the spike. The relations between the applied current intensity and the spike amplitude and its maximum rate of rise were sigmoidal. The maximum rate of rise of spike was increased up to 20 v/sec by the inward current, but it remained at this maximal value during application of an even stronger stimulus intensity. The application of tetrodotoxin (5×10^{-7} g/ml.) had no influence on the augmentation of the spike amplitude caused by hyperpolarizing current.

In *Loligo* giant nerve fibre (Hodgkin & Huxley, 1952), the changes of the maximum rate of rise of spike are related to the membrane potential. Since the bridge method used in our experiments does not give a reliable value for the membrane potential and since

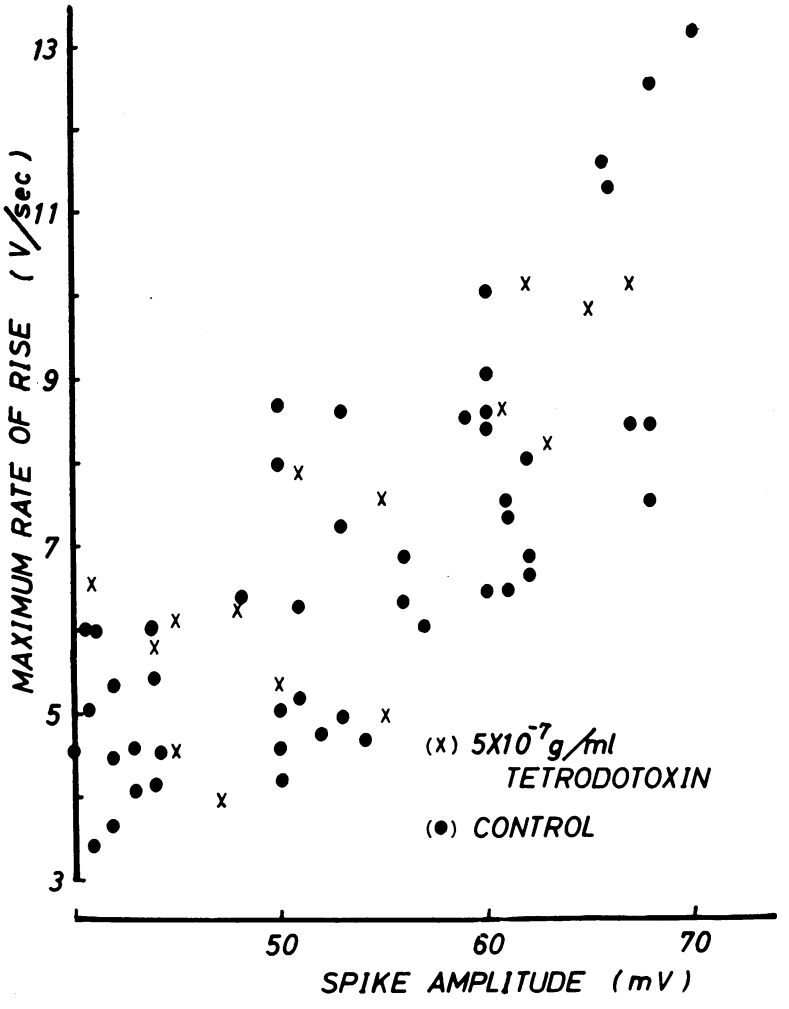

Fig. 4. Guinea-pig taenia coli. Relationship between the spike amplitude and the maximum rate of rise of spike before (●) and after (X) treatment with tetrodotoxin (5×10^{-7} g/ml.) at various amplitudes of the action potential caused by conditioning polarizing current.

the spike amplitude is proportional to the membrane potential, it is possible to use the relation between the maximum rate of rise of the spike and spike amplitude (Kuriyama & Tomita, 1965). Fig. 4 shows the relationship between the maximum rate of rise and various amplitudes of the action potential during conditioning polarization before and after treatment with tetrodotoxin (5×10^{-7} g/ml.). No effect of tetrodotoxin on the above relationship could be observed.

In a few cells, intracellularly applied outward (depolarizing) current triggered a spike. The response was mostly graded and only rarely an "all or none" spike. Tetrodotoxin (5×10^{-7} g/ml.) had no effect on this response. Stimuli of subthreshold intensity applied

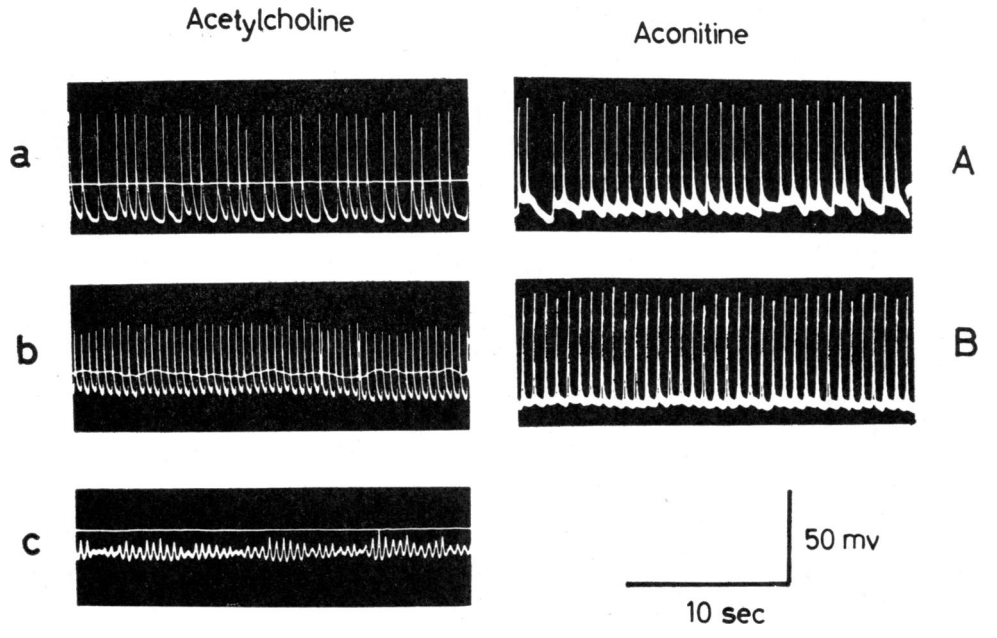

Fig. 5. Effects of aconitine (10^{-6} g/ml.) on the smooth muscle cell membrane of guinea-pig taenia coli, in comparison with that of acetylcholine (10^{-7} g/ml.). Acetylcholine blocked the spike generation by profound depolarization of the cell membrane, but aconitine had much less effect on the spontaneous activity of the cell membrane. (a) Control. (b) Acetylcholine after 2 min. (c) After 10 min. (A) Control. (B) Aconitine after 10 min.

intracellularly produced electrotonic potential changes, from which the effective resistance and time constant could be measured. They were 30–90 MΩ, and 1.8–6.8 msec respectively. These values agreed with those obtained by Kuriyama & Tomita (1965). Tetrodotoxin had no influence on the above parameters.

Effect of tetrodotoxin on the membrane activity of smooth muscle in excess calcium and sodium-free solution

When the tissue was soaked in high calcium solution (7.5 mM–25 mM) which was buffered by Tris (tris(hydroxymethyl)-aminomethane), the spontaneous activity ceased but extracellularly applied electrical current could elicit a spike. Even in this condition, 10^{-7} g/ml. tetrodotoxin had no effect on the amplitude and the maximum rate of rise of the spike.

When the Na ion was replaced with Tris (titrated with HCl, and *p*H adjusted to 7.3), the membrane was transiently hyperpolarized to about 70 mV and the spontaneous spike activity stopped. After a quiescent period of 5–10 min the membrane became gradually depolarized and spikes of regular amplitude reappeared. The spike activity continued for 40 min, but never longer (Bülbring & Kuriyama, 1963). However, the maximum rate of rise of the spike gradually decreased in sodium deficient solution. Tetrodotoxin (5×10^{-7} g/ml.) had no effect on the above phenomena and their time course.

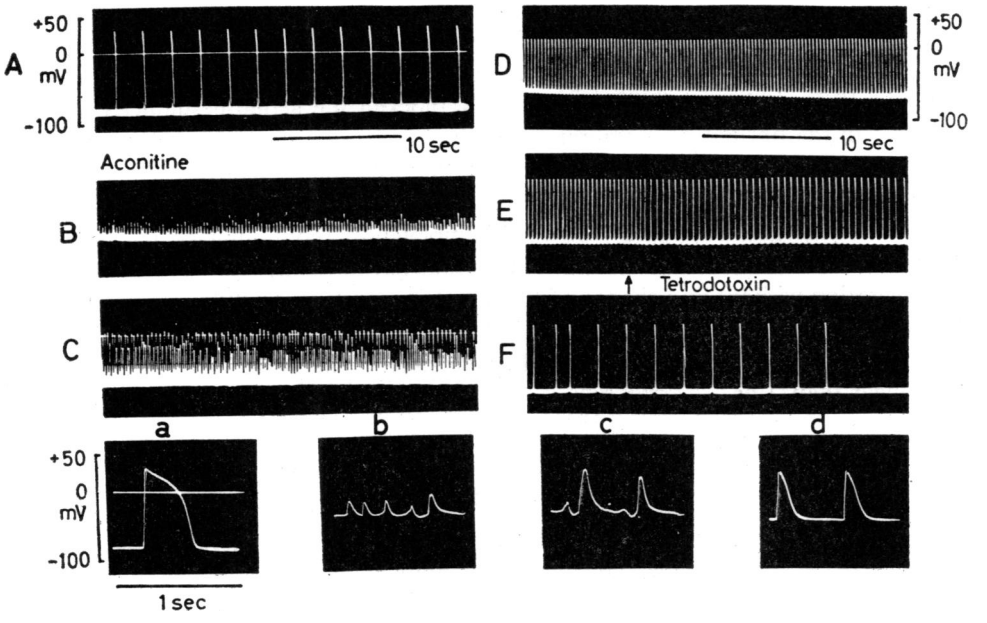

Fig. 6. Effect of aconitine (10^{-6} g/ml.) on the papillary muscle of guinea-pig heart. (A) control; spikes were triggered by electrical stimulation. (B) After 5 min treatment with aconitine; aconitine induced fibrillation of membrane. (C) After long washing in Krebs solution; the membrane activity changed from fibrillation to flutter. (D) typical pattern of flutter. (E) and (F) treatment with tetrodotoxin (10^{-7} g/ml.); tetrodotoxin reduced the frequency of spontaneously generated spikes and finally blocked the spike generation without any change of the membrane potential. (a) to (d) show fast records of the action potential corresponding to those of (A) to (D).

Effect of tetrodotoxin on smooth muscle, cardiac muscle and skeletal muscle after pre-treatment with aconitine

In the present experiments aconitine was diluted to 10^{-6} g/ml. in the organ bath while the flow of the Krebs solution was stopped for about 5 min. The aconitine was then washed out by starting the flow of the modified Krebs solution without aconitine. After this exposure to aconitine the smooth muscle cell membrane was depolarized by about 5 mV and the spike frequency was increased to 1.2–1.5 times the normal frequency. Fig. 5 shows the effect of aconitine in comparison with that of acetylcholine, which, like aconitine, is believed to increase the ionic permeabilities of chemically excitable cell membranes. The effect of aconitine in increasing the membrane activity was less than that of acetylcholine. Tetrodotoxin (10^{-6} g/ml.) had no effect on the membrane activity accelerated by aconitine or acetylcholine.

In contrast with such a weak action of aconitine on smooth muscle, it had a remarkable effect on cardiac muscle and skeletal muscle.

Aconitine (10^{-6} g/ml.) significantly depolarized the cell membrane of the auricular and the papillary muscles and often produced depolarization block of the spike generation. After washing out the aconitine with Krebs solution, the tissues again generated spikes described as aconintine-induced fibrillation or flutter. Fig. 6 shows a typical effect on

the papillary muscle of the guinea-pig during the treatment with aconitine. After treatment with aconitine, the membrane activity was accelerated to "flutter," then to fibrillation, and after long exposure to Krebs solution the membrane activity again showed "flutter"; 10^{-7} g/ml. tetrodotoxin completely blocked the aconitine-induced fibrillation or flutter. In the fibrillating membrane, the above concentration of tetrodotoxin reduced the spike frequency, changing fibrillation to flutter, and finally blocked the spike generation. When the concentration of tetrodotoxin was increased to 5×10^{-7} g/ml., the fibrillation of the membrane was rapidly blocked completely without showing the intermediate pattern of "flutter." In the "flutter" condition, tetrodotoxin (10^{-7} g/ml.) blocked the spike generation without any change of the membrane potential. When the concentration of tetrodotoxin was lower than 5×10^{-7} g/ml. and its application time was short, its effect on the membrane activity was completely reversible. The effect of aconitine, however, lasted longer than 2 hr.

DISCUSSION

In the smooth muscle, tetrodotoxin did not block the spike generation but sometimes increased the spike frequency. Furthermore, both at the normal membrane potential level and in the hyperpolarized condition caused by inward electrical current or excess calcium, tetrodotoxin had no influence on the membrane activity. Moreover, in sodium-deficient solution, tetrodotoxin had no effect on the membrane activity.

The failure of tetrodotoxin to abolish the action potential in smooth muscle may throw doubt on earlier interpretations which assumed a poorly developed and partially inactivated sodium carrying system, and rather supports the assumption that in the spike generation of smooth muscle Ca^{++} may take the place of Na^{+}. The observation that the maximum rate of rise of the action potential is proportional to the external Ca^{++} concentration has not only been made in the taenia coli (Bülbring & Kuriyama, 1963) but also by many authors in other excitable cells (Fatt & Ginsborg, 1958; Greengard & Straub, 1959; Tasaki, 1959; Werman & Grundfest, 1961; Hagiwara & Naka, 1964; Hagiwara, Chichibu & Naka, 1964). Hagiwara & Naka (1964) discussed the role of Ca^{++} during the active phase of the membrane of barnacle muscle fibres and stated that calcium ions not only had a stabilizing action on the membrane (Frankenhäuser & Hodgkin, 1957) but also that the ions entering during the spike were Ca^{++}. Furthermore, they observed that tetrodotoxin had no effect on the spike generation mechanism.

In contrast to smooth muscle, skeletal muscle and cardiac ventricle were highly sensitive to low concentrations of tetrodotoxin. When "fibrillation" or "flutter" had been produced by aconitine, tetrodotoxin completely blocked the spike generation of the papillary muscle of the heart. These observations may indicate that aconitine increased the ionic permeabilities of dominantly electrically sensitive cell membranes and that tetrodotoxin prevented the increase of the sodium permeability during the active state. Furukawa, Sasaoka & Hosoya (1959) reported that tetrodotoxin had no effect on the neuromuscular junction, indicating that tetrodotoxin had no effect on dominantly chemically sensitive cell membranes. In fact, the smooth muscle is influenced neither by tetrodotoxin nor by aconitine, but strongly influenced by acetylcholine.

From the present experiments, it is rather difficult to decide whether calcium or sodium carries the charge during the active state of the membrane in such a chemically excitable cell membrane. It is only possible to conclude that the spike generation mechanism of the smooth muscle cell is qualitatively different from that of the skeletal muscle cell. A decisive interpretation might be obtained only by the voltage clamp method.

SUMMARY

1. The effects of tetrodotoxin (10^{-8}–5×10^{-6} g/ml.) were observed on the membrane activities of smooth muscle, cardiac muscle and skeletal muscle of the guinea-pig.

2. Tetrodotoxin had no effect on the membrane activities of taenia coli and the pacemaker cells of the heart but sometimes increased the spike frequency. In contrast, tetrodotoxin blocked the spike generation without any change of the membrane potential in ventricular muscle of the heart and skeletal muscle of the diaphragm.

3. Intracellularly applied hyperpolarizing currents increased the amplitude and maximum rates of rise and fall of the spike of the smooth muscle cell of taenia coli, the relationship between the maximum rate of rise and the spike amplitude being sigmoidal. Tetrodotoxin had no effect on these phenomena.

4. Excess calcium hyperpolarized the membrane and increased the amplitude and the maximum rate of rise of the spike. Tetrodotoxin had no effect on the changes of the membrane activities caused by excess calcium.

5. Aconitine (10^{-8}–5×10^{-6} g/ml.) slightly increased the spike frequency and depolarized the membrane of the smooth muscle cell; tetrodotoxin did not modify the effect of aconitine. In contrast, aconitine produced fibrillation or flutter in the muscle cells of the heart and the diaphragm; tetrodotoxin blocked spike generation also in the presence of aconitine.

6. The observations suggest that the spike generation mechanism of the smooth muscle cell is qualitatively different from that of the skeletal and the cardiac ventricular muscle cells. The possible mechanism of spike generation in the smooth muscle cell is discussed.

REFERENCES

BÜLBRING, E. (1957). Changes in configuration of spontaneously discharged spike potentials from smooth muscles of the guinea-pig taenia coli. The effect of electrotonic currents and of adrenaline, acetylcholine and histamine. *J. Physiol.* (*Lond.*), **135**, 412–425.

BÜLBRING, E., HOLMAN, M. & LÜLLMAN, H. (1956). Effects of calcium deficiency on striated muscle of the frog. *J. Physiol.* (*Lond.*), **133**, 101–117.

BÜLBRING, E. & KURIYAMA, H. (1963). Effects changes in the external sodium and calcium concentrations on spontaneous electrical activity in smooth muscle of guinea-pig taenia coli. *J. Physiol.* (*Lond.*), **166**, 29–58.

FATT, P. & GINSBORG, B. L. (1958). The ionic requirements for the production of action potentials in crustacean muscle fibres. *J. Physiol.* (*Lond.*), **142**, 516–543.

FLEISHER, J. H., KILLOS, P. J. & HARRISON, C. S. (1961). Effects of puffer poison on neuromuscular transmission. *J. Pharmac. exp. Ther.*, **133**, 98–105.

FRANKENHÄUSER, B. & HODGKIN, A. L. (1957). The action of calcium on the electrical properties of squid axons. *J. Physiol.* (*Lond.*), **137**, 218–244.

FURUKAWA, T., SASAOKA, T. & HOSOYA, Y. (1959). Effects of tetrodotoxin in the neuromuscular junction. *Jap. J. Physiol.*, **9**, 143–152.

GOTO, M., TAMAI, T. & YANAGA, T. (1963). Studies on the appearance and termination of aconitine-induced atrial fibrillation with microelectrodes. *Jap. J. Physiol.*, **13**, 196–207.

GRAHAM, HELEN T. & GASSER, H. S. (1931). Modification of nerve response by veratrine, protoveratrine and aconitine. *J. Pharmac. exp. Ther.*, **43**, 163–185.

GREENGARD, P. & STRAUB, R. W. (1959). Restoration by barium of action potentials in sodium deprived mammalian B and C fibres. *J. Physiol. (Lond.)*, **145**, 562–569.

HAGIWARA, S. & NAKA, K. (1964). The initiation of spike potential in barnacle muscle fibres under low intracellular Ca^{++}. *J. gen. Physiol.*, **48**, 141–162.

HAGIWARA, S., CHICHIBU, S. & NAKA, K. (1964). The effects of various ions on resting and spike potentials of barnacle muscle fibres. *J. gen. Physiol.*, **48**, 163–179.

HERZOG, W. H., FEIBEL, R. M. & BRYANT, S. H. (1964). The effect of aconitine on the giant axon of the squid. *J. gen. Physiol.*, **47**, 719–733.

HODGKIN, A. L. & HUXLEY, A. F. (1952). Current carried by sodium and potassium ions through the membrane of the giant axon of Loligo. *J. Physiol. (Lond.)*, **116**, 449–472.

HOLMAN, M. E. (1958). Membrane potentials recorded with high-resistance micro-electrodes and the effects of changes in ionic environment on the electrical and mechanical activity of the smooth muscle of the taenia coli of the guinea-pig. *J. Physiol. (Lond.)*, **141**, 464–488.

KURIYAMA, H. & TOMITA, T. (1964a). Effect of polarizing currents on the membrane activity of single smooth muscle cells. *J. Physiol. (Lond.)*, **173**, 10–11P.

KURIYAMA, H. & TOMITA, T. (1964b). Electrical responses of single smooth muscle cells to intracellular electrical stimulation. *J. Physiol. (Lond.)*, **175**, 36–37P.

KURIYAMA, H. & TOMITA, T. (1965). The responses of single smooth muscle cells of guinea-pig taenia coli to intracellulary applied current, and their effect on the spontaneous electrical activity. *J. Physiol. (Lond.)*, **178**, 270–289.

MATSUDA, K., HOSHI, T. & KAMEYAMA, S. (1959). Effect of aconitine on the cardiac membrane potential of the dog. *Jap. J. Physiol.*, **9**, 419–429.

NAKAMURA, T., NAKAJIMA, S. & GRUNDFEST, H. (1965). The action of tetrodotoxin on electrogenic components of squid giant axons. *J. gen. Physiol.*, **48**, 985–996.

NARAHASHI, T. (1964). Restoration of action potential by anodal polarization in lobster giant axons. *J. cell. comp. Physiol.*, **64**, 73–96.

NARAHASHI, T., DEGUCHI, T. URAKAWA, N. & OHKUBO, Y. (1960). Stabilization and rectification of muscle fiber membrane by tetrodotoxin. *Am. J. Physiol.*, **93**, 318–336.

NARAHASHI, T., Moore, J. W. & Scott, W. R. (1964). Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. *J. gen. Physiol.* **47**, 965–974.

SCHERF, D. (1947). Studies on auricular tachycardia caused by aconitine administration. *Proc. Soc. exp. Biol. Med.*, **64**, 233–239.

SCHERF, D., SCHAEFFER, A. I. & BLUMENFELD, S. (1953). Mechanism of flutter and fibrillation. *A. M. A. Archs. intern. Med.*, **91**, 333–352.

TAMAI, T., YANAGA, T. & GOTO, M. (1961). On the transmission of excitation in the atrial muscle during aconitine-induced fibrillation. *Jap. J. Physiol.*, **11**, 656–665.

TASAKI, I. (1959). Resting and action potentials of reversed polarity in frog nerve cells. *Nature (Lond.)*, **184**, 1574.

TOIDA, N. & OSA, T. (1965). International Congress of Physiological Sciences XXIII, Tokyo; Abstracts, p. 94.

WERMAN, R. & GRUNDFEST, H. (1961). Graded and all-or-none electrogenesis in arthropod muscle. II. The effects of alkaliearth and onium ions on lobster muscle fibers. *J. gen. Physiol.*, **44**, 997.

WOODBURY, J. W. & BRADY, A. J. (1956). Intracellular recording from moving tissues with a flexibly mounted microelectrode. *Science*, **123**, 100–101.