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ELECTROPHYSIOLOGICAL STUDIES ON EMBRYONIC HEART CELLS IN CULTURE

SCORPION TOXIN AS A TOOL TO REVEAL LATENT FAST SODIUM CHANNEL

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Summary

Trypsin-dispersed heart cells were obtained from 11-day-old chick embryos. After culture as unstirred suspensions in dimethylsulfoxide-containing medium, spherical aggregates of cells beating spontaneously and apparently synchronously for months were obtained. Two kinds of cell were characterized by electrophysiological recordings: (1) cells with a slow rate of depolarizing phase showing tetrodotoxin-resistant action potential and blocked by D 600 ('slow' cells); (2) cells with high value of rising phase which was strongly decreased by tetrodotoxin and in which D 600 provoked uncoupling of excitation-contraction ('fast' cells).

Toxin II from *Androctonus australis* scorpion venom increased the duration of action potential, which was ascribed to a slowing down of Na^+ current inactivation and enhance the maximum rate of depolarization, especially in slow cells. Effects were antagonized by tetrodotoxin in both fast and slow cells. Washing experiments confirmed the results of previous studies, namely that tetrodotoxin and scorpion toxin bind to different receptors. It is concluded that slow cells with tetrodotoxin-resistant action potential contain latent fast Na^+ channels that are revealed (activated) by toxin binding to the membrane.

Abbreviations: TC, tissue cultured; NTC, non-tissue cultured; Me_2SO, dimethylsulfoxide.

Introduction

In spite of extensive investigations, the mechanism of ion transport across membranes at the molecular level is still unknown. Drugs influencing specifically the closing or the opening of ion channels are potentially important tools for the analysis of electrogenesis in nerve and heart. In this respect, the polypeptide neurotoxins from the venom of the scorpion *Androctonus australis* Hector were shown to affect reversibly the closing of the Na^+ channel and the opening of the K^+ channel in nerve membranes of crustaceans [1]. Using electrophysiological methods [2], similar results were obtained in electrically excitable neuroblastoma cells in culture. Embryonic heart cells which maintain autorhythmic activity in culture also offer a useful material to study the ionic channels that control excitability of heart membrane. They show the electrical and mechanical properties of embryonic and adult heart [3,4].

The effects of toxin II from *A. australis* venom on the adult heart had been studied previously [5]. On cultured chick embryonic heart cells it provoked chronotropic and inotropic effects [6] and stimulated the passive uptake of Na^+ and Ca^{2+} at concentrations as low as 10 nM [7]. The present studies were undertaken to describe the effects of *A. australis* toxin II on the electrical properties of membranes of cultured chick embryonic heart cells and to compare the results with those obtained previously by ion transport methods [7]. Culture in appropriate conditions in the presence of dimethylsulfoxide allowed the heart cell to maintain automaticity for long periods of time and to permit prolonged electrophysiological recordings.

Methods and Materials

Cell cultures

Isolated cells were obtained from hearts of 11-day-old chick embryos by the trypsinization procedure as previously described [6]. Cells were suspended at a concentration of $1.0—1.5 \cdot 10^6$ cells/ml in NCTC 109 medium containing 10% calf serum, 200 units/ml penicillin G and 50 μg/ml streptomycin sulfate, and were seeded in Falcon plastic flasks (not treated for tissue culture). Cultures in unstirred suspensions were maintained at 35° C in a humidified atmosphere of air/CO_2 (95 : 5). At day 3, the culture medium was removed by centrifugation (150 $\times g$ for 5 min) and replaced by fresh medium containing 2% dimethylsulfoxide. Incubation at 35° C was continued and this could be done up to several weeks provided medium change to fresh medium containing Me_2SO was performed twice a week.

For electrical recording, 2 ml of cell suspension were seeded in plastic Petri dishes (treated for tissue culture) and incubation was continued under the same conditions. The cell aggregates formed during the period of culture in unstirred suspension settled and, as early as 3 h after transfer into Petri dishes, they adhered sufficiently to the substratum to be impaled by the microelectrode. Characteristics of culture conditions will be referred to in the legends of figures and in the text as NTC or TC, followed by a number to indicate number of days of culture in suspension (NTC) or as adhering aggregates (TC). Cells seeded in Petri dishes for 3 h only, will be referred to as TC 0.

Electrical recordings

Before and during impalement, cells were superfused with Dulbecco's modified Eagle's medium buffered with 20 mM HEPES containing 1% fetal calf serum, 2% Me_2SO and, when indicated, drugs to be tested. Superfusion was carried out through capillary bridges at a flow rate of 0.5—1.5 ml/min. The turnover rate constant was 0.5 min^{-1} for a flow of 1 ml · min^{-1}. 50 and 90% of the final concentration of additives in the dish medium were reached in about 1.5 and 5 min, respectively, as estimated by checking with ^{125}I-labeled scorpion toxin. Temperature of the bath was 36 ± 0.5°C. Cells were impaled using glass microelectrodes with an inner filament (Clark, E.I., Pangbourne, U.K.) filled with 3 M KCl. Tip diameter was less than 0.3 μm and resistance measured in 3 M KCl was 20—40 MΩ. Signal was recorded through a high input impedance (greater than 20 GΩ) preamplifier with capacitance neutralization. The preamplifier was inserted in a bridge circuit (WP Instr. mod. 701, New Haven, U.S.A.) so that depolarizing current pulses could be applied through the voltage-recording electrode especially to test pacemaker activity of the cells. The signal and its first-time derivative were recorded on a storage oscilloscope (Tektronics 50II N) and photographed.

Materials

Toxin II from *A. australis* Hector venom was purified according to Miranda et al. [8]. Chemicals were obtained from the following sources: NCTC 109 medium from Eurobio (Paris, France); Dulbecco's modified Eagle's medium and fetal calf serum from Gibco (Paisley, U.K.); tetrodotoxin and HEPES from Sigma (St. Louis, MO, U.S.A.); dimethylsulfoxide from Merck (Darmstadt, F.R.G.); D 600 (5-methoxy-(α-isopropyl-α(*N*-methyl-*N*-homoveratryl)-γ-aminopropyl)-3,4-dimethoxyphenylacetonitrile hydrochloride) from Knoll Laboratories (Ludwigshafen, F.R.G.); veratridine from EGA-Chemie (Steinheim, F.R.G.); tetraethylammonium chloride from BDH (Poole, U.K.).

Results

1. Membrane electrical properties of chick embryonic heart cells

In this study, the culture of chick embryonic heart cells in the presence of Me_2SO was investigated in order to avoid changes in some membrane electrical properties that have been assigned to a possible loss of differentiation due to the conditions of culture. Indeed, Me_2SO was shown to induce the differentiation of leukaemic cells infected with Friend virus [9] and of neuroblastoma cells [10] in culture. In fact, culture in the presence of Me_2SO resulted in inconsistent effects on membrane electrical properties but allowed the embryonic heart cells to maintain automaticity for up to 3 months of cultivation time.

Electrical recordings were all made on spontaneously beating cell clusters. As far as could be ascertained by microscope observation, all cells in an aggregate beat synchronously. No difference in resting potential and maximum value of spike potential was observed between cells cultured in the presence or in the absence of Me_2SO.

78 cells were impaled using 69 preparations. In a few experiments, the mem-

brane potential rapidly dropped after impalement, but, routinely, action potentials could be recorded with the same electrode for 1—3 h. Table I summarizes values of the electrical parameters which were measured systematically: resting potential, E_r; potential at maximum value of the spike, E_p; duration of action potential at half-maximum value of repolarization, D_{50}; and maximum rate of rise of action potential, $+\dot{V}$. The average resting potential (—56 mV), average action potential (+30 mV) and average duration of action potential (190 ms) are in general agreement with those reported by other investigators [3,4,11].

In contrast to that which should had been expected from the previously described dependence of $+\dot{V}$ on resting potential in cultured embryonic heart cells [12], the plot of values of $+\dot{V}$ against E_r (Fig. 1) showed a distribution of cells into two distinct populations with $+\dot{V} \leqslant 10\ V \cdot s^{-1}$ and E_r in the range —40 to —62 mV, and with $+\dot{V} \geqslant 15\ V \cdot s^{-1}$ and E_r between —40 and —72 mV. Cells with high value of $+\dot{V}$ showed a significant and close relationship between $+\dot{V}$ and E_r, whereas cells with low values of $+\dot{V}$ disclosed no dependence of $+V$ on E_r.

These two kinds of cell will be referred to further as slow ($+\dot{V} \leqslant 10\ V \cdot s^{-1}$) and fast ($+\dot{V} \geqslant 15\ V \cdot s^{-1}$) cells. To avoid equivocal results, only fast cells with $+\dot{V} \geqslant 60\ V \cdot s^{-1}$ have been used to explore the effect of drugs. Table II summarizes the electrical characteristics of the two kinds of cell selected.

Fast and slow cells also responded differently to tetrodotoxin exposure (Fig. 2 and Table III). At 0.4 μM, the toxin had no influence on E_r and $+\dot{V}$ of slow cells and on E_r of fast cells but produced a strong (about 90%) and highly significant decrease of $+\dot{V}$ of fast cells (Table III). This effect was rapidly reversed by washing (Fig. 2B).

An origin for the difference between slow and fast cells was sought in the conditions used for cell culturing.

High values of $+\dot{V}$ (greater than or equal to $60\ V \cdot s^{-1}$) were always observed in cells cultured firstly for 7—21 days in suspension (NTC 7—21) and then as adhering aggregates in Petri dishes from 2—10 days (TC 2—TC 10). Low values of $+\dot{V}$ (less than or equal to $10\ V \cdot s^{-1}$) were recorded in cells at all ages of cultivation. In some conditions of culture (TC 2 and TC 3), the dishes con-

TABLE I

SOME ELECTRICAL PARAMETERS OF CULTURED CHICK EMBRYONIC HEART CELLS

	Mean ± S.E.	Range	Number of determinations
Resting potential (E_r) *	—56.7 ± 0.7 mV	—40 to —78	78
Potential at maximum value of the spike (E_p)	+29.6 ± 0.8 mV	+16 to +42	78
Duration of action potential (D_{50}) **	190.7 ± 3.6 ms	120 to 268	78
Maximum rate of depolarization ($+\dot{V}$)	30.1 $V \cdot s^{-1}$ ***	3 to 125	76

* Resting potential was easily determined in non-pacemaker cells as the level from which the rapid upstroke was initiated and not as often utilized the maximum diastolic potential. Value at the onset of prepotential was chosen in pacemaker cells with an error lower than 2 mV.

** Duration at 50% repolarization.

*** All values between 3 and 125 $V \cdot s^{-1}$ were found with a non-gaussian distribution. About 50% of cells (42/76) disclosed a $+\dot{V} \leqslant 10\ V \cdot s^{-1}$.

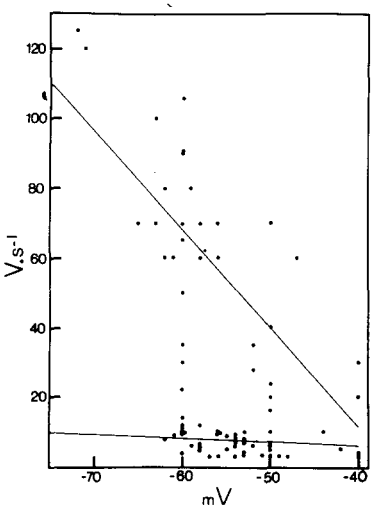

Fig. 1. Relationship between rate of rise of action potential and resting potential in cultured embryonic heart cells. Electrical parameters of cells with $+\dot{V} \leqslant 10$ V · s^{-1} (n = 42) are: $+\dot{V}$ = 6.90 ± 0.45 V · s^{-1}; E_r = —54.10 ± 0.75 mV. Equation of the linear regression line (power line) is: $+\dot{V}$ = —0.18 E_r —3.51 with r = 0.37, not significant ($P < 0.05$). Electrical parameters of cells with $+\dot{V} \geqslant 15$ V · s^{-1} (n = 32) are: $+\dot{V}$ = 60.19 ± 5.24 V · s^{-1}; E_r = —57.09 ± 1.30 mV. Equation of linear regression line (upper line) is: $+\dot{V}$ = —2.83 E_r —101.6 with r = 0.70, highly significant ($P < 0.0001$). Difference between the means of values for the two kinds of cell is significant with $P < 0.0001$ for $+\dot{V}$ and $P < 0.01$ for E_r.

tained a mixture of cells with high and low $+\dot{V}$ so that it was difficult to predict the type of cell to be met under any particular condition of cultivation.

In our conditions, the highest value of $+\dot{V}$ recorded (125 V · s^{-1}) was lower than in other studies (up to 200 V · s^{-1}). However, in the latter, cultured cells were obtained from 16-day-old chick embryonic hearts [13,14] whose rising phase of action potential was steeper [15].

2. *Effects of scorpion toxin*

At concentrations below 3 nM, scorpion toxin was without effect on mem-

TABLE II

ELECTRICAL PARAMETERS AND PACEMAKER ACTIVITY OF SLOW AND FAST CHICK EMBRYONIC HEART CELLS

	Number of measurements	$+\dot{V}$ (V · s^{-1})	E_r (mV)	E_p (mV)	D_{50} (ms)	Pacemaker activity
All cells together	78	30.1	—56.7 ± 0.7	+29.6 ± 0.8	190.7 ± 3.6	55/78
Slow cells	42	6.9 ± 0.4 *	—54.1 ± 0.7 **	+26.4 ± 1.0 ***	197.8 ± 4.1 **	40/42
Fast cells	20	78.5 ± 4.1 *	—60.4 ± 1.3 **	+30.7 ± 1.6 ***	168.3 ± 6.2 **	6/20

Results expressed as means ± S.E.:

* Significant difference ($P < 0.0001$) by paired t-test.

** Significant difference ($P < 0.005$) by paired t-test.

*** Nonsignificant difference ($P < 0.01$) by paired t-test.

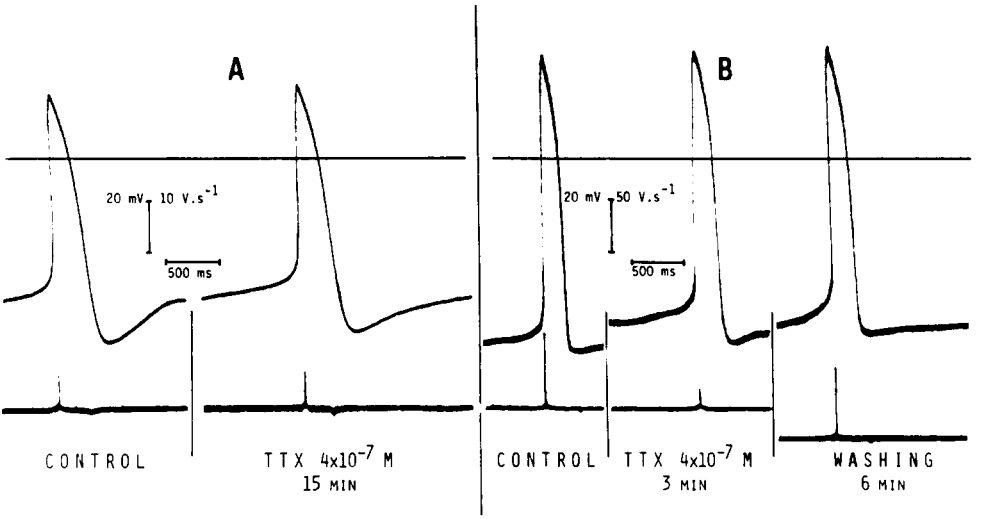

Fig. 2. Effects of tetrodotoxin on the action potential of spontaneously beating slow and fast cells. A, no effect of 0.4 μM tetrodotoxin (TTX) on a slow cell (NTC 11; TC 0) even after 15 min exposure to the toxin; B, 3 min after the onset of superfusion with tetrodotoxin-containing medium, $+\dot{V}$ is strongly reduces in a fast cell (NTC 11; TC 3). Effect is reversed by washing in drug-free medium. In all figures the upper line shows the zero value of membrane potential, the medium line the intracellular potential record (V) and the lower line the time derivative (dv/dt) of the potential V.

brane electrical parameters and cell beating. At concentrations higher than 0.1 μM, fibrillation and arrhythmia occurred which made it difficult to keep the microelectrode inside the cell. At 30 nM, large effects were always observed and since cell impalement could be maintained for 1 h or more, this concentration was used in most experiments.

Effects of scorpion toxin on the electrical parameters of chick embryonic heart cells are collected in Table IV. The time course of its action on slow and fast cells is illustrated in Fig. 3.

The major effect was an approximately 10-fold increase in duration of the

TABLE III

ACTION OF TETRODOTOXIN ON MAXIMUM RATE OF DEPOLARIZATION AND RESTING POTENTIAL OF SLOW AND FAST CELLS

		Slow cells (n = 6)	Fast cells (n = 4)
Before TTX	E_r (mV)	53.33 ± 2.9	63.25 ± 2.9
	$+\dot{V}$ (V · s^{-1})	6.33 ± 1.3	71.50 ± 3.0
After TTX (0.4 μM)	E_r (mV)	51.83 ± 3.5	56.50 ± 2.1
	$+\dot{V}$ (V · s^{-1})	6.50 ± 1.4	8.25 ± 1.9
	ΔE_r	—1.50 ± 0.05 *	—6.75 ± 1.60 **
	$\Delta +\dot{V}$	—0.17 ± 0.07 *	63.75 ± 2.69 ***

Values are means ± S.E.

* Nonsignificant difference ($P < 0.10$) by paired t-test.

** Nonsignificant difference ($P < 0.01$) by paired t-test.

*** Significant difference ($P < 0.001$) by paired t-test.

TABLE IV

EFFECT OF TOXIN II FROM *A. australis* VENOM ON THE ELECTRICAL PARAMETERS OF SLOW AND FAST CHICK EMBRYONIC HEART CELLS

Values are means ± S.E. Recordings were done 8 min after the onset of superfusion with medium containing 30 nM toxin II.

	Addition to medium	E_r (mV)	E_p (mV)	$+\dot{V}$ ($V \cdot s^{-1}$)	D_{50} (s)
Slow cells ($N = 14$)	None	−55.8 ± 1.4 *	+28.3 ± 1.3 *	8.6 ± 0.6 **	195.3 ± 6.6 **
	Toxin II, 30 nM	−53.6 ± 2.0 *	+29.2 ± 1.6 *	36.8 ± 2.0 **	786.5 ± 57.2 **
Fast cells ($N = 10$)	None	−61.1 ± 2.0 *	+30.4 ± 1.8 *	73.5 ± 5.2 *	172.6 ± 8.2 **
	Toxin II, 30 nM	−59.8 ± 2.2 *	+30.3 ± 2.1 *	82.7 ± 6.8 *	583.8 ± 74.9 **

* Nonsignficant difference ($P < 0.10$) by paired *t*-test.
** Significant difference ($P < 0.0001$) by paired *t*-test.

action potential. In some experiments using scorpion toxin II concentrations higher than 30 nM, the lengthening was so large that the membrane potential remained near −10 mV and cell beating stopped. This effect occurred in slow (Fig. 3A) as well as in fast cells (Fig. 3B) and it was also observed in cells with intermediate rate of rise (Fig. 4B). A very slight and not significant depolarization was also noticed in both kinds of cell without modification of peak value of the spike (E_p).

While no difference was observed between fast and slow cells in their E_r, E_p and D_{50} response to scorpion toxin, the maximum rate of rise of the action potential was affected differently in each type of cell. In slow cells, a large increase of $+\dot{V}$ occurred (Fig. 3A). Its mean value (37 $V \cdot s^{-1}$) was about 4-times greater in the presence of 30 nM scorpion toxin than in its absence (9 $V \cdot s^{-1}$). Nevertheless, $+\dot{V}$ never reached values as high as in fast cells (74 $V \cdot s^{-1}$). In fast cells, enhancement of $+\dot{V}$ also occurred (Fig. 3B) but to a lesser (and not significant) degree than in slow cells (Table IV).

Reversal to control values occurred slowly and was generally nearly complete after washing for 20—30 min with drug-free medium (Fig. 5A). However in some experiments, only partial reversibility was observed (Fig. 3) which was accompanied by the maintaining of the $+\dot{V}$ increase.

As described above, tetrodotoxin had a very small effect on the resting potential and overshoot of both kinds of cell and only reduced the maximum rate of rise of fast cells. In contrast to this poor effect on normal cells, tetrodotoxin in all cases rapidly suppressed scorpion toxin II-induced modifications. When tetrodotoxin (0.1 μM, final concentration) was added to the scorpion toxin II-containing superfusion medium, enhancement of duration and rate of rise of the action potential disappeared (Fig. 4A) and return to normal action potential was complete in less than 6 min after the onset of tetrodotoxin perfusion. In fast cells, the specific effect of tetrodotoxin in strongly reducing $+\dot{V}$ was observed. In the cases where washing with drug-free medium was unable to reverse completely the scorpion toxin II effect, this could be obtained immediately by washing with tetrodotoxin-containing medium (Fig. 3B). When a slow cell was perfused with both scorpion toxin and tetrodotoxin, its action poten-

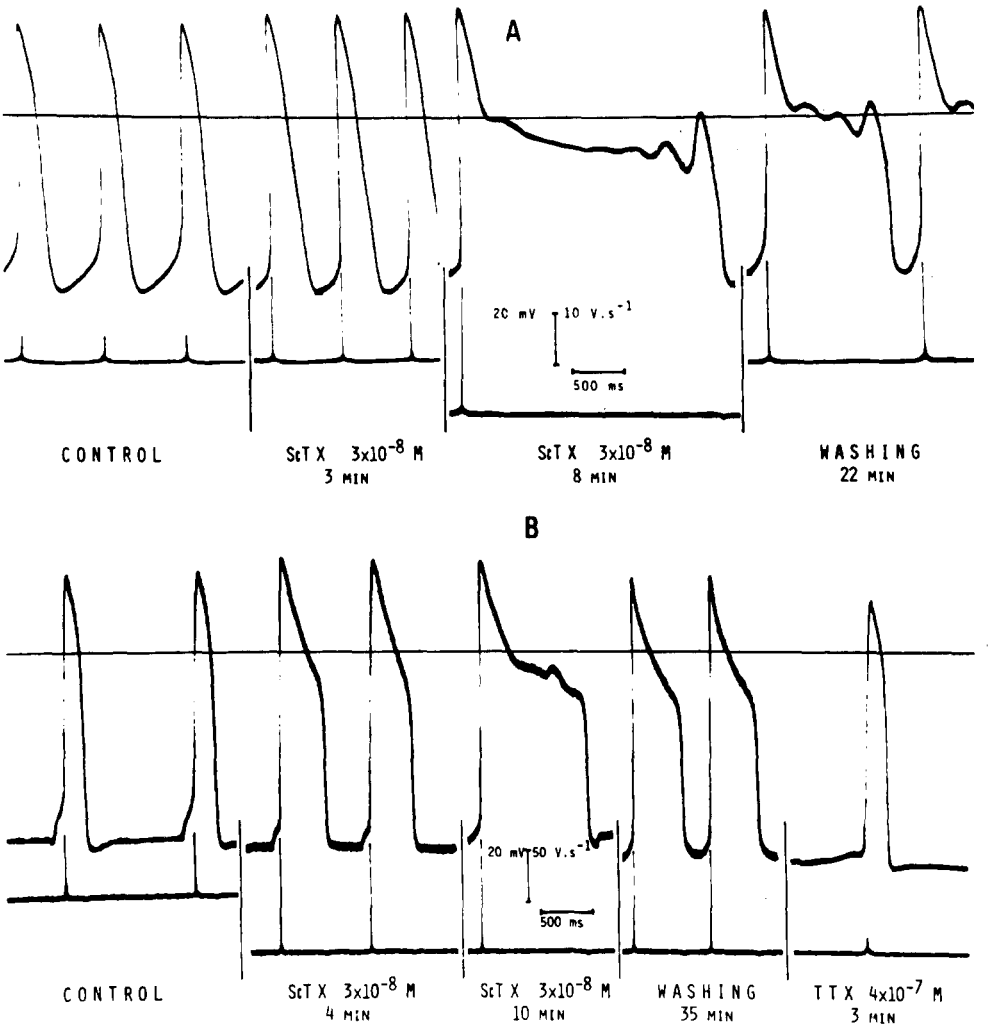

Fig. 3. Effects of scorpion toxin ScTX (30 nM) on the action potential and its time derivative. A, a rapid increase of $+\dot{V}$ occurred and consecutively a lengthening of duration of action potential. Incomplete reversal of the toxin effect after washing for 22 min in drug-free medium (conditions of culture: NTC 11; TC 0). B, incomplete reversal of scorpion toxin effect after 35 min washing in the drug free medium and rapid effect of washing with tetrodotoxin-containing medium (TTX) (NTC 7; TC 4).

tial (and time derivative) appeared 'normal'. However, cell washing in drug-free medium rapidly restores the scorpion toxin-induced effect on D_{50} and $+\dot{V}$ (Fig. 4B).

3. Effects of scorpion toxin and D 600

D 600, a calcium channel blocking drug acting specifically on the slow Ca^{2+} channel abolishes the contractile response of cardiac muscle without producing appreciable changes in Na^+-dependent action potential [11,16]. At a concentration of 0.1 μM, D 600 blocks beating of cultured chick embryonic heart cells in monolayer [6].

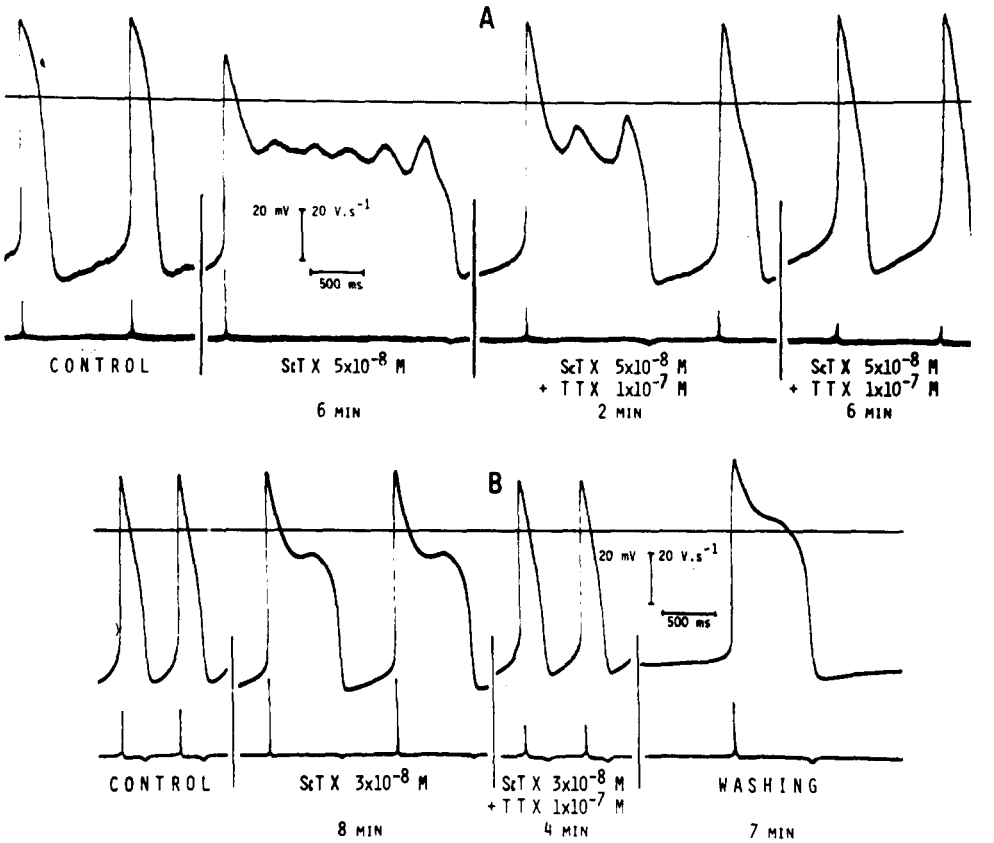

Fig. 4. Effect of tetrodotoxin on scorpion toxin-induced modifications of action potential. A, from the 2nd min of superfusion with scorpion toxin II (ScTX) and tetrodotoxin (TTX), the scorpion toxin effect begins to disappear. B, washing with drug-free medium for 7 min evidences unmasking of the scorpion toxin effect previously reversed by tetrodotoxin. (NTC 4; TC 0).

Superfusion of slow cells in aggregate, with 0.5 μM D 600 induced in some cases a transient phase of action potential firings without beats; then a weak depolarization with membrane potential oscillations occurred (Fig. 5B). At an higher concentration of D 600 (1 μM), beats and firings stopped immediately. When scorpion toxin (30 nM) was added on slow cells treated by 0.5 μM D 600, beats and firings reappeared in the scorpion toxin mode. Addition of tetrodotoxin (0.1 μM) immediately suppressed the scorpion toxin effect and reversal of the effect produced in the presence of D 600 alone was observed (Fig. 5B).

When fast cells were perfused with 1 μM D 600, suppression of beats also occurred, but normal action potential (Fig. 5C) were easily recorded for a long time (uncoupling of excitation-contraction). However with higher concentrations of D 600, a decrease of membrane electrical activity also occurred in fast cells. As in slow cells, addition of scorpion toxin resulted in a typical scorpion toxin action potential (Fig. 5C) but, in this case, addition of tetrodotoxin not only induced the suppression of scorpion toxin effect, but also the abolition of firings which were not previously suppressed by D 600.

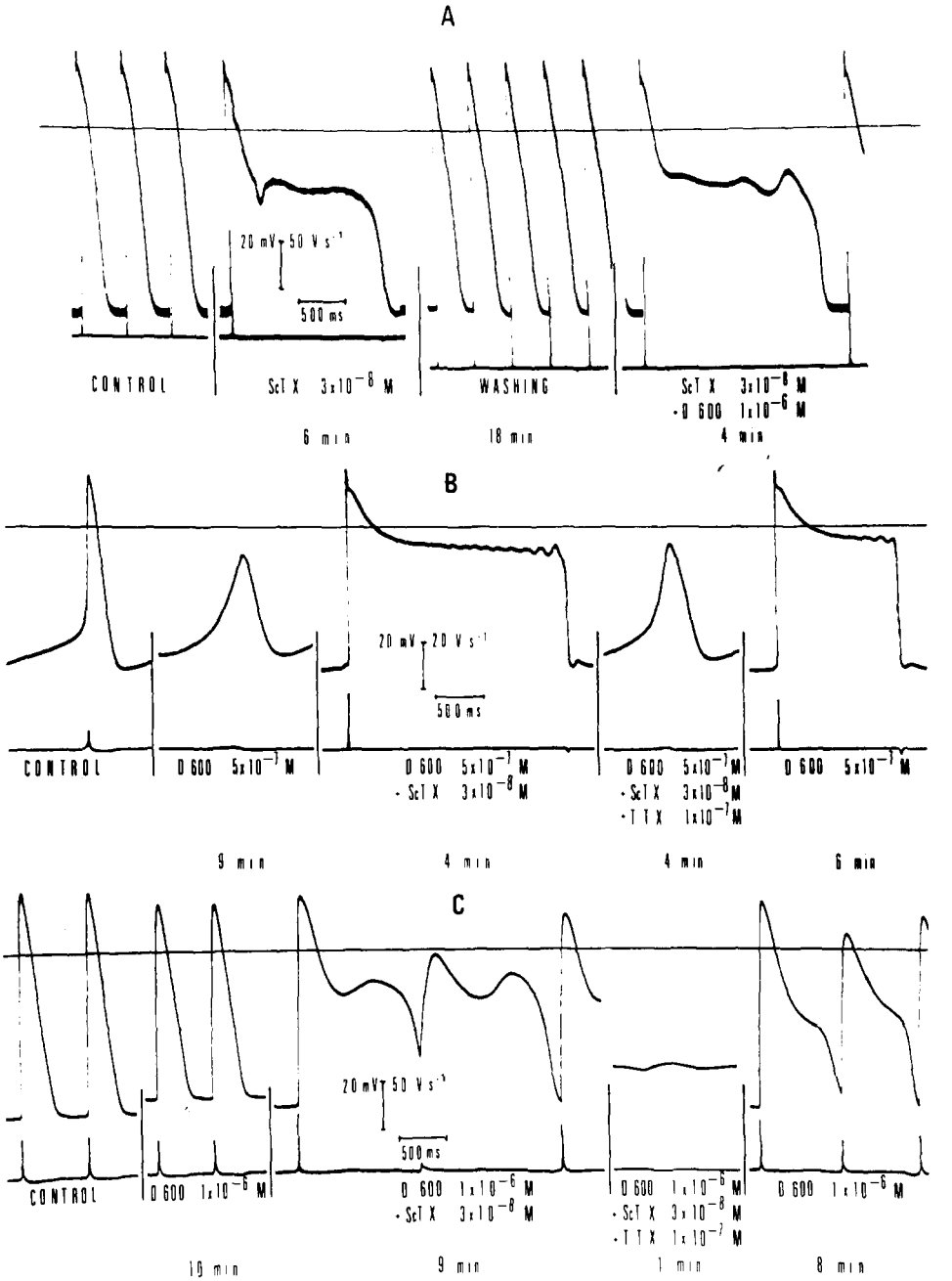

Fig. 5. Effects of scorpion toxin and D 600 on the action potential. A, effect of 30 nM scorpion toxin on a fast cell and complete reversal with washing. In experiments where D 600 (1 μM) was present together with scorpion toxin (ScTX), the same large toxin effect occurred more quickly. (NTC 17; TC 10). B, D 600 (0.5 μM) abolishes cell beatings and drastically reduces the amplitude of the spike and $+\dot{V}$ (slow cell). Addition of scorpion toxin reverses D 600 effect and scorpion toxin effect appears. Supplementation with tetrodotoxin results in the reversal of scorpion toxin effect and return to action potential in the presence of D 600. With washing in D 600-containing medium, scorpion toxin effect reappears (NTC 3; TC 0). C, D 600 (1 μM) abolishes cell beating without affecting action potential firing on fast cells (excitation-contraction uncoupling). Abolition of beating was studied by microscopic observation of the cells. Addition of scorpion toxin induces a characteristic scorpion toxin effect. Addition of tetrodotoxin immediately stops firing and membrane potential remains near its resting value. 8 min after washing (D 600 medium), action potential reappears with typical scorpion toxin shape (NTC 8; TC 5).

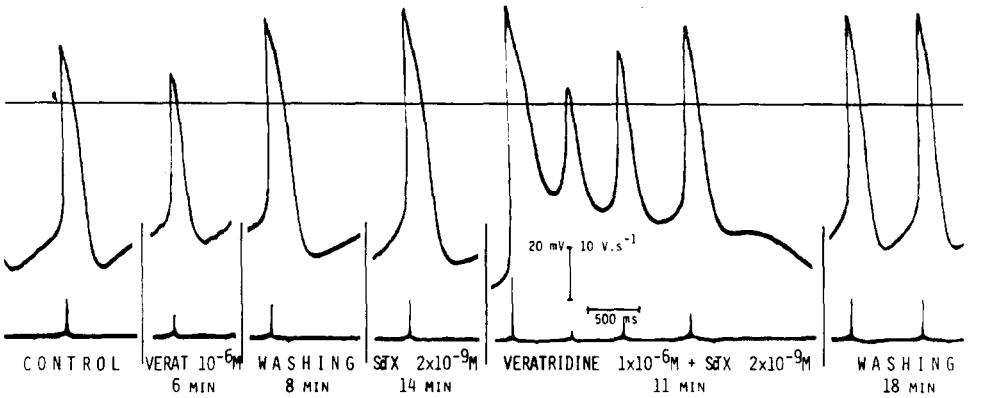

Fig. 6. Effects of veratridine (Verat) and scorpion toxin (ScTX) on action potential. Scorpion toxin (2 nM) is without effect on the action potential. Addition of 1 μM veratridine to the scorpion toxin-containing medium results in significant atypical modifications. This effect is reversed by washing (NTC 11; TC 0).

In both cases, washing the cells with D 600-containing medium induced the reappearance of the scorpion toxin effect (Fig. 5B and 5C).

4. Effects of scorpion toxin and other compounds

The alkaloid veratridine causes repetitive action potentials and in some cases, persistent depolarization of nerves [17] and cardiac cells [18]. It modifies both activation and inactivation of the fast Na^+ channel [17], but its binding site on the membrane appears to be different from that of scorpion toxin [19]. At concentrations lower than 0.5 μM, veratridine has no effect on the action potential of cultured embryonic heart cells; at 1 μM, a small depolarization and a decrease of the amplitude of the action potential occur. When veratridine was added to medium together with a concentration of 2 nM scorpion toxin (which by itself does not induce action potential modification), a scorpion toxin effect was observed, i.e., enhancement of $+\dot{V}$ and of duration of the repolarization phase of the spike but in an atypical mode with superimposed repetitive action

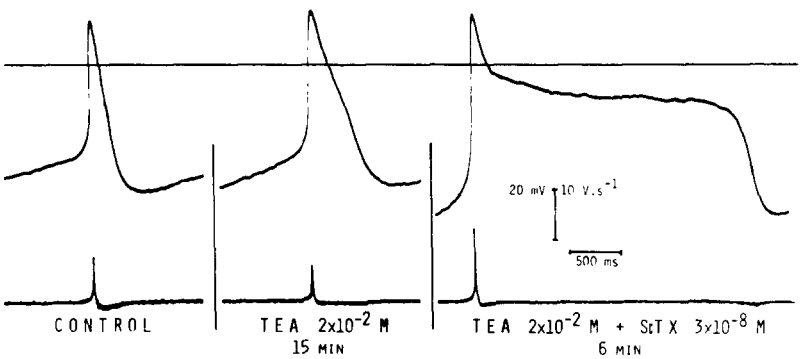

Fig. 7. Effects of tetraethylammonium (TEA) and scorpion toxin on action potential. Prolongation of duration of the action potential is 7-times greater with scorpion toxin plus tetraethylammonium than with tetraethylammonium alone.

potentials and a slow fall to resting potential. Both veratridine and scorpion toxin effects disappeared upon washing in normal medium (Fig. 6).

Tetraethylammonium is an inhibitor of the membrane conductance of K^+ [17]. In adult rat heart it enhances the duration of action potential [20] as in embryonic rat heart [21]. At a concentration of 20 mM tetraethylammonium, the same effect occurred in chick embryonic heart cells in culture (Fig. 7), i.e., prolongation of the duration of the action potential with slowing down of the final phases of repolarization.

When scorpion toxin was added to tetraethylammonium-treated cells, prolongation of duration of the plateau phase was 7-fold greater than with tetraethylammonium alone, the last phase of repolarization remaining of the tetraethylammonium type.

Discussion

Trypsin-dispersed chick embryonic heart cells have been cultured as unstirred suspensions in medium containing Me_2SO. In these conditions, spontaneously beating spherical aggregates can be maintained for months without alteration of membrane electrical properties. Cells cultured in the presence of Me_2SO showed values of resting potential and overshooting similar to those recorded from cells cultured in its absence. The average values of membrane electrical parameters were also in general agreement with those reported by other investigators in cultured chick embryonic heart cells [3,4]. However, in the present experiments, the maximum rate of depolarization changed from cell to cell and values of $+\dot{V}$ from 3—125 $V \cdot s^{-1}$ were recorded. Causes for these differences can be sought in the mechanism of electrogenesis in cardiac cells. In adult heart of various species, the rising phase of the action potential can be resolved into a fast and a slow component, each of them being associated with separate inward currents. The fast inward current is carried by Na^+, is blocked by tetrodotoxin and activated near 15 mV in voltage-clamp experiments [22,23]. The slow component is due to a current of Na^+ and Ca^{2+}; it is insensitive to tetrodotoxin, blocked by Mn^{2+} and specific organic compounds such as verapamil and D 600 [16,23] and activated at an higher voltage than fast current; it can trigger firing and electromechanical coupling in Na^+-free medium [24,25] but not under normal ionic conditions. The slow inward current plays a major role in producing the plateau phase of the action potential [20,22]. Repolarization of the membrane is due to both a Cl^- [26] and a K^+ current [23] and to inactivation of the Na^+ current.

During embryonic development, important modifications of intracellular ionic composition and membrane permeability to K^+ in the heart [27] and of sensitivity to drugs have been observed. In young hearts (less than day 4), action potential is generated by slow Na^+ channels which confer slow rates of rise of action potential [15] and which are insensitive to tetrodotoxin, a specific blocker of fast Na^+ channels. In older hearts, from about 5 days in chick [3,28] and 11 days in rat [21], Na^+ channels are fast and tetrodotoxin-sensitive. However, it was shown [29—31] that cells dispersed from old hearts and cultured in monolayer became rapidly tetrodotoxin-insensitive and showed slow Na^+ channels characteristic of younger embryonic state. The mechanism

of reversion from fast to slow Na^+ channels in embryonic heart cells in culture is still unknown. Nevertheless, the conditions of heart cell interaction [11,32—35], the composition of the culture medium [29,36] or the nature of the substratum used for cell cultivation [13,37] appeared to be important factors in maintaining the presence of tetrodotoxin-sensitive fast Na^+ channels.

Under our conditions of culture, about 7 days of incubation in suspension and at least 2—3 days of post-culture as adhering cells were required to obtain spontaneously beating cells with high values of rate of rise. However, low values of $+\dot{V}$ were observed with all times of culture. Although the reason(s) for the observed heterogeneity of the rising phase of the action potential is still unknown, it is clear that tetrodotoxin-sensitivity of our cultured heart cells is correlated with high values of $+\dot{V}$, i.e., to the presence of fast Na^+ channels.

The two kinds of cell manifest the following properties. Slow cells contains Na^+ channels insensitive to tetrodotoxin but sensitive to Ca^{2+}-blocking drugs such as D 600. They are generally of the pacemaker type. In contrast, tetrodotoxin is effective in fast cells as shown by $+\dot{V}$ decrease and D 600 induces at low concentration excitation-contraction uncoupling. They are often devoid of pacemaker activity.

In these cells, scorpion toxin induced a sustained prolongation of duration of the action potential without appreciable modifications of the resting potential, as previously described in nerve axons [38—40], neuroblastoma cells [2], rat heart [5] or tunicate eggs [41]. Voltage-clamp studies in myelinated axons showed that scorpion venom markedly affected the inactivation process of Na^+ conductance [39,40] and this was confirmed with pure scorpion toxin in non-myelinated axons [1]. Tetrodotoxin which specifically blocks fast Na^+ channels, antagonized the action of scorpion toxin in cultured heart cells, so that a rapid reversal of scorpion toxin effect was observed using both electrophysiological (this paper) and ion transport [7] methods. In other respects, rates of release of toxin effects as shown by washing experiments (Fig. 4B) confirmed previous findings [7] that tetrodotoxin and scorpion toxin bind to different receptors on the heart cell membrane.

Scorpion toxin was also shown to decrease the steady state K^+ current [1,38,39]. However, this effect does not occur in all membranes [41]. In cultured heart cells from 11-day-old chick embryo, the K^+ exchangability was very low [27] so that it is not surprising that the effect of scorpion toxin occurred even when the K^+ channel was blocked by tetraethylammonium (Fig. 7).

In our studies, scorpion toxin was shown to provide an efficient tool with which to gain information on Na^+ ionophores in cultured embryonic heart cells. Indeed, the reversal of scorpion toxin action by tetrodotoxin was observed in fast cells and also in slow cells whose action potential is generated by tetrodotoxin-insensitive slow Na^+ channels. Thus, slow cells also contain fast Na^+ channels that are unmasked by scorpion toxin binding to the membrane. This finding confirms previous conclusions using ion transport methods that scorpion toxin may unmask latent tetrodotoxin sensitive channels in embryonic cardiac cell in culture [7] and, more recently, the presence of tetrodotoxin receptors in embryonic heart cells displaying a tetrodotoxin-resistant action potential as revealed by the alkaloid neurotoxin veratridine [42]. However,

methods using ion transport to measure membrane permeability to ions are unable to determine whether the two kinds of Na^+ channel are actually present in a single cell or in two populations of cells. The present studies using electrophysiological methods ascertain that the former possibility is most probable.

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