Possible Existence of Transient TTX-Sensitive Chloride Current in the Membrane of Isolated Cardiomyocytes

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Abstract. Cardiomyocytes enzymatically isolated from rat and guinea pig ventricular tissue were investigated under conditions of intracellular perfusion and voltage clamp at 18—20 °C. Perfusion with 135 mmol/l Tris(HF), pH 7.2 was used to eliminate outward potassium currents. The dependence of inward current (elicited by depolarizing pulses from a holding potential level of — 120 mV) on low external TTX concentrations (from 10^{-13} to 10^{-10} mol/l) was studied. Similar TTX concentrations increased the amplitude of the inward current and changed its kinetics in a large number of cells tested. The effect was fully reversible. The effect could be evaluated in a net form by digital subtraction of the current obtained after the application of a low external TTX concentration from the initial current in a TTX-free solution. The TTX concentration dependence of the difference current could be fitted by one-to-one binding curve with $K_d = (1.0 \pm 0.4) \times 10^{-12}$ mol/l. TTX-induced current changes were absent in low sodium or chloride-free external solutions. The outward current (a block of which by TTX produced the inward current changes observed) showed a reversal potential consistent with the chloride nature of such a current. The existence of a transient TTX-sensitive Na-dependent potential gated chloride current in the membrane of isolated cardiomyocytes is postulated.

Key words: Cardiomyocytes — Tetrodotoxin — Chloride — Current

Introduction

The use of isolated Ca^{2+}-tolerant heart muscle cells in electrophysiological experiments allowed a considerable progress in the understanding of ionic mechanisms underlying the excitability of the cardiac tissue (Irisawa 1984; Reuter 1984; Fozzard et al. 1985).

Generally, the use of a new object in a new experimental environment can help to evaluate new features of the behaviour of ionic currents. A newly observed phenomenon, namely the effect of picomolar external tetrodotoxin concentrations on ionic currents of single cardiomyocytes is the topic of the present paper, with an attempt to evaluate the ionic nature of the phenomenon observed.

Materials and Methods

The techniques used in the present experiments (cell isolation, voltage clamp, intracellular perfusion and the exchange of extracellular solutions) were the same as described elsewhere (Kostyuk et al. 1981; Pidoplichko 1983; 1986). Experiments were carried out mainly on single rat cardiomyocytes. Several experiments were performed also on cardiomyocytes of one month old guinea pigs. Identical techniques were used for both types of experiments. The compositions of the solutions used in the present investigations were as follows (in mmol l). Extracellular solutions: 1) NaCl — 154; KCl — 5; $Ca(NO_3)_2$ — 3.6; $Mg(NO_3)_2$ — 1.1; MOPS — 10; adjusted to pH 7.4 with Tris — OH 2) NaCl — 160; $Ca(NO_3)_2$ — 3.6; MOPS — 10; adjusted to pH 7.4 with Tris — OH. Methanesulphonic acid anions were substituted for chloride ions in Cl^--free or low-chloride solutions; it is known that methanesulphonic anion is one of the best substitutes for chloride ions in electrophysiological experiments (Horackova and Vassort 1982; Barish 1983). Tris ions were substituted for cations if necessary. The calcium concentration was maintained standard in all external solutions using a Ca^{2+}-selective electrode (Ingold, model 9320, Switzerland).

Tetrodotoxin (Serva, FRG; Sakyo, Japan) was diluted with deionised water from a 1×10^{-2} mmol l stock solution to obtain basic concentrations values from 10^{-8} to 10^{-11} mol l. The required TTX concentrations were obtained by adding corresponding amounts of basic solutions into experimental chambers (1 ml volume each).

Intracellular solutions contained (in mmol l): 1) Tris(HF) — 135; pH 7.2, 2) Tris(HF) — 70; NaCl — 65; pH 7.2, 3) $Tris(H_2PO_4)$ — 135; pH 7.2

Results

After the substitution of the artificial intracellular saline for intracellular milieu, inward currents were elicited by depolarizing command potential pulses delivered with 1 Hz frequency from a holding potential of — 120 mV. The inactivation decay of the evoked currents could not be fitted by one exponential, the currents observed being obviously of a complex (multicomponent) nature. The complex current observed under initial conditions shall be referred to as the "total inward current".

The application of external solutions with low (from 10^{-13} to 10^{-10} mol/l) TTX concentrations to the membrane of perfused isolated cardiomyocytes induced a marked increase of the amplitude of the total inward current along

with slowing down its inactivation decay (Fig. 1). A similar effect was observed in more than 50 % of the cells tested. The action of low TTX concentrations was fully reversible: the washout of TTX resulted in a complete restoration of the initial parameters (i.e. a decrease and speeding up of the current; Fig. 1*c*). Digital subtraction of the current record obtained with 10^{-11} mol/l TTX from the initial current (recorded in TTX-free solution) yielded the tetrodotoxin-sensitive component in the net form (Fig. 1*d*). The decay of this current component (it shall be referred to as the "difference current") could be well fitted by one exponential with a time constant (τ_{diff}) of about 17 ms for the testing potential (V_t) value of -20 mV (Fig. 1*d*).

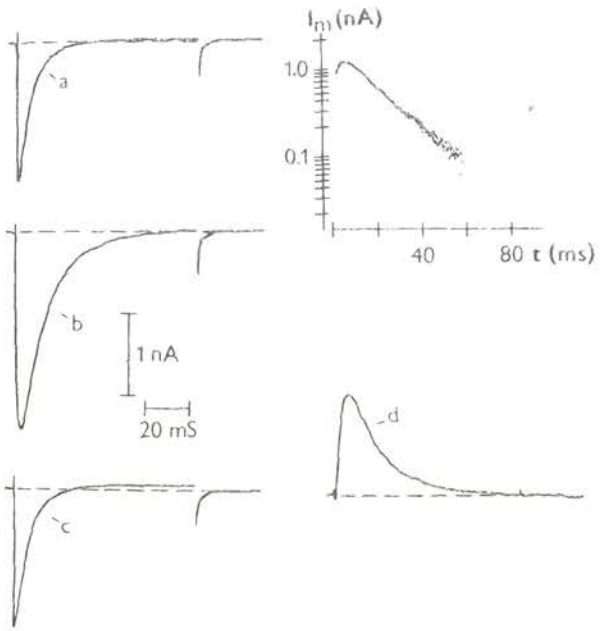

Fig. 1. The effect of 10^{-11} mol/l TTX on the total inward current of isolated rat cardiomyocyte. *a*: initial record in TTX-free extracellular solution; *b*: the increase and the change in the current kinetics due to the action of 10^{-11} mol/l TTX; *c*: washout of the TTX action in normal extracellular solution; *d*: the difference current, obtained by digital subtraction of trace *b* from trace *a*. In the upper right part of the figure, the difference current (*d*) is shown in a semilogarithmic scale ($\tau_{diff} = 17$ ms). The time scale is the same for all traces. $V_h = -120$ mV; $V_t = -20$ mV; sampling rate 5 kHz; intracellular solution 1.

The substitution of nonpermeable cations ($Tris^+$) for K^+ and Mg^{2+} in normal extracellular solution did not alter the TTX effect. Further experiments were therefore carried out in external solution "2" (see Materials and Methods). The effect remained unaltered also when fluoride ions in the intracellular perfus-

ing solution (intracellular solution "1") were replaced by phosphate ions (intracellular solution "3") and vice versa.

The increase in the total inward current amplitude in the presence of picomolar TTX concentrations was potential dependent and could be observed over a V_t range from -65 to $+40$ mV (Fig. 2). The increase in the current amplitude varied from several to several hundred percents for different cells. In some instances the increase in the current amplitude was so strong that it resulted in a loss of potential control in voltage clamp conditions: the appearence of a very steep initial part on the I—V curve and a fast artifact component in the current decay. Such cases are illustrated in Fig. 3. It should be stressed that to ensure good voltage control the experiments were carried out mainly on small cells which displayed considerably small (after TTX-induced increase) currents with amplitudes of several nA. The difference current inactivation decay could be described by one exponential under the above conditions only. Some of the cells showed almost no increase in the current amplitude at low TTX concentrations but a considerable change in the total inward current inactivation decay kinetics (Fig. 4). These observations (Fig. 3, 4) may be explained by superposition of different permeability components which have different weights in different cells.

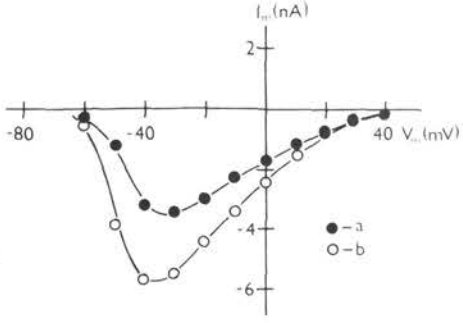

Fig. 2. I—V curve for the total inward current in TTX-free solution (*a*) and I—V curve for the inward current in 10^{-11} mol l TTX (*b*). Experiment on a single rat ventricular myocyte. $V_h = -120$ mV; intracellular solution 1.

Owing to this the quantitative estimation of the effect of picomolar TTX concentrations based on changes in inward current amplitude would not be correct in all cases. Consequently changes in the "difference current" (i.e. in the current obtained after digital substraction of the current in TTX-containing solutions from the corresponding total inward current in tetrodotoxin-free solution) were analysed.

The current-voltage relationship (I—V curve) of the difference current had a maximum around V_t -40 mV (Fig. 5). Moreover, the difference current showed a dependence on V_h (with steady-state inactivation), and it disappeared completely at V_h more positive than -70 mV. The dependence of the difference current on external tetrodotoxin concentrations could be well fitted by

the one-to-one binding curve (Fig. 6) with $K_d = (1.0 \pm 0.4) \times 10^{-12}$ mol/l ($n = 9$).

Dependence of the effect of low TTX concentrations on the ionic composition of extra- and intracellular solutions

In order to evaluate the ionic nature of changes in membrane permeability induced by picomolar TTX concentrations a series of experiments was carried out.

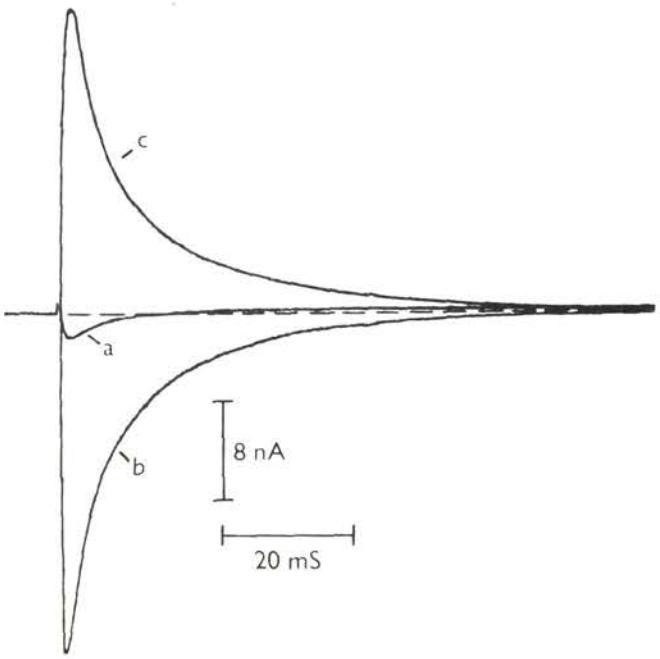

Fig. 3. The dramatic effect of 10^{-10} mol/l TTX on the total inward current of a single guinea pig ventricular myocyte. *a*: initial current trace in TTX-free solution (the amplitude corresponds to about I_{max}); *b*: a drastic increase of the current induced by 10^{-10} mol/l TTX; *c*: the result of digital subtraction of trace *b* from trace *a*. Similar pronounced increase of the current amplitude resulted in a poor voltage control. $V_h = -125$ mV; $V_t = -30$ mV; sampling rate 5 kHz; intracellular solution 1.

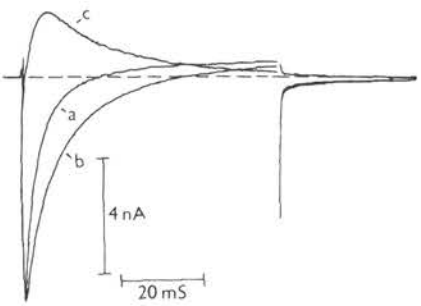

Fig. 4. An example of a practical lack of the increase of the current amplitude following 10^{-10} mol/l TTX; only a change in the kinetics was observed. *a*: current in TTX-free solution; *b*: changes induced by TTX; *c*: difference current trace (the inactivation time constant of the difference current is about 18 ms). Experiment on a single guinea-pig ventricular myocyte. $V_h = -120$ mV; $V_t = -20$ mV; sampling rate 5 kHz; intracellular solution 1.

In cardiomyocytes responding to low TTX concentrations, a slight decrease in the sodium content of the external solution (from 160 to 130 mmol/l) induced a paradoxical increase in the total inward current amplitude and a slowing down of its decay (Fig. 7). TTX (10^{-10} mol/l) had no effect in the solution containing 130 mmol/l Na_o^+ (Fig. 7*B*, traces b, c). After the wash out in TTX-free solution with 160 mmol/l Na_o^+, the current parameters returned to the initial values (i.e. the current amplitude diminished and the decay was enhanced). At the same time, the current (which increased after the application of 10^{-10} mol/l TTX — trace a, Fig. 7*C*) decreased (trace b, Fig. 7*C*) as soon as the sodium content was lowered.

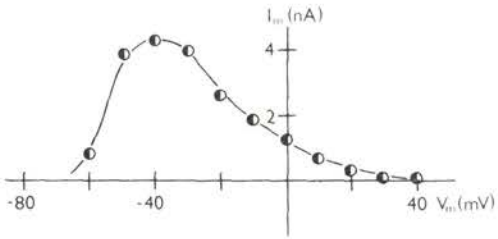

Fig. 5. I—V curve for peak difference current. Experiment on a single rat ventricular myocyte. $V_h = -120$ mV.

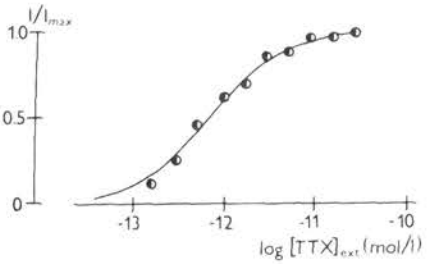

Fig. 6. The concentration dependence of the difference current increase on low extracellular TTX concentrations (normalized). The concentration dependence is well fitted by one-to-one binding curve (solid line) with $K_d = 10^{-12}$ mol/l $[TTX]_o$. Abscissa: external TTX concentration, logarithmic scale. Experiment on a single rat ventricular myocyte. $V_h = -120$ mV; $V_t = -20$ mV; intracellular solution 1.

A possible explanation is that a slight lowering of the sodium content in the external solution has a strong cooperative effect on the same mechanism which is affected by low doses of tetrodotoxin.

A decrease of the chloride ion concentration in the external solution resulted in a decrease of the TTX effect (Fig. 8), i.e. in a decrease of the difference current amplitude. The kinetics of the difference current decay was not affected by the procedure. A complete substitution of nonpermeable anions for chloride in the external solution abolished the effect of low TTX concentrations on the ionic currents of the membrane of cardiomyocytes (no current changes, no "difference current" in Cl^--free solution, — Fig. 8*c*). It is worth mentioning that in cells

which responded to low TTX concentrations an increase and a slowing down of the decay of the total inward current was observed after their transfer from the initial, chloride-containing to Cl^--free solution (Fig. 9).

Hence the effect of picomolar TTX concentrations on inward currents of isolated perfused cardiomyocytes appeared to be Na^+- and Cl^--dependent; definite conclusions concerning the ionic nature of the phenomena observed can therefore hardly be drawn. Further experiments were designed to bring more information on the issue.

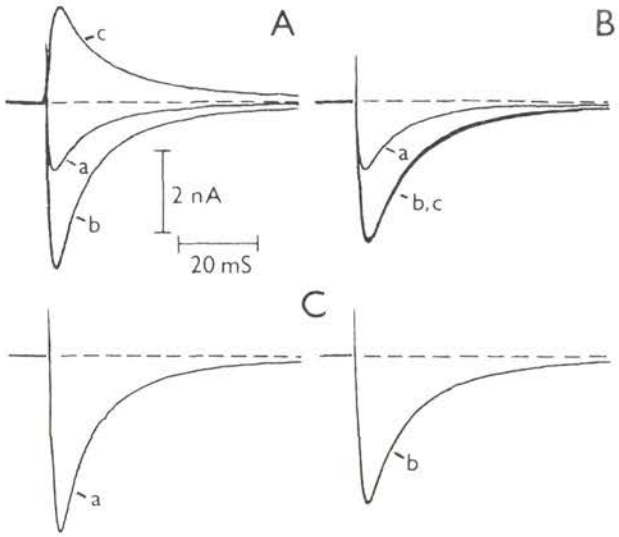

Fig. 7. Sodium dependence of the difference current. *A*. *a*: initial current in normal TTX-free extracellular solution; *b*: 10^{-10} mol/l TTX; *c*: the difference current (obtained by digital subtraction of trace *b* from trace *a*). *B*. Current increase due to the lowering of the extracellular sodium concentration from 160 mmol/l (trace *a*) to 130 mmol/l (trace *b*). After the application of 10^{-10} mol/l TTX to 130 mmol/l $[Na^+]_0$ solution, the current trace (*c*) coincided with trace *b*. *C*. Lowering of extracellular sodium in solutions containing 10^{-10} mol/l TTX. *a*: the current in TTX containing extracellular solution (160 mmol/l Na^+); *b*: the current in TTX containing extracellular solution (130 mmol/l Na^+). Experiment on a single rat ventricular myocyte. $V_h = -120$ mV; $V_t = -20$ mV; sampling rate 5 kHz; intracellular solution 1.

A solution containing 65 mmol/l NaCl (intracellular solution "2") was substituted for the intracellular Tris-fluoride solution. The extracellular solution contained 160 mmol/l Na^+ and 160 mmol/l Cl^-. It can be assumed that under similar experimental conditions, the reversal potential for the difference current should reveal its ionic nature (theoretical values for E_{NA^+} and E_{Cl^-} for the concentrations mentioned above are +22.5 and −22.5 mV, respectively). The current-voltage relationship for the difference current showed the reversal poten-

tial around −27 mV, which is in agreement with the theoretical value for E_{Cl} = −22.5 mV (Fig. 10). In other words, at V_t lower than −27 mV, 10^{-10} mol/l TTX would induce a decrease of the inward current amplitude, and at V_t more positive than −27 mV the inward current would increase.

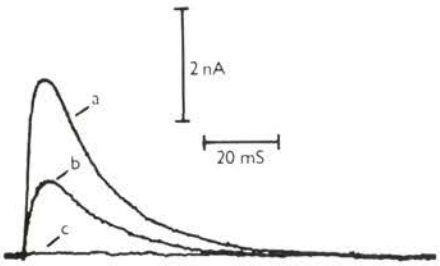

Fig. 8. The dependence of the TTX-sensitive difference current on the extracellular chloride concentration. *a*: difference current in 160 mmol/l $[Cl^-]_0$; *b*: difference current in 80 mmol/l $[Cl^-]_0$; *c*: lack of difference current in Cl^--free extracellular solution. Experiment on a single rat ventricular myocyte. V_h = −120 mV; V_t = −20 mV; sampling rate 5 kHz; intracellular solution 1.

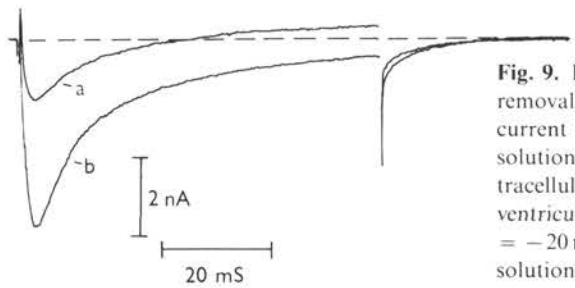

Fig. 9. Inward current changes due to chloride removal from extracellular solution. *a*: inward current in normal Cl^--containing extracellular solution; *b*: inward current in Cl^--free extracellular solution. Experiment on a single rat ventricular myocyte. V_h = −120 mV; V_t = = −20 mV; sampling rate 5 kHz; intracellular solution 1.

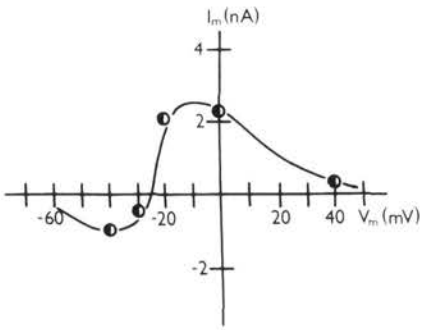

Fig. 10. I—V curve of the TTX-sensitive difference current (plotted for peak difference current values); estimation of the reversal potential. E_{Cl^-} = −22.5 mV; E_{rev} = −27 mV. Experiment on a single rat ventricular myocyte. V_h = = −120 mV; intracellular solution 2.

The influence of external pH on the difference current

Further analysis of the properties of the difference current (i.e. of the ionic permeability component revealed by the application of low external TTX concentrations) showed a strong dependence of the difference current on external

pH. Changes of external pH from 7.4 to 6.8, or from 7.4 to 8.2, induced a complete block of the difference current (Fig. 11). The experimental points in Fig. 11, representing difference current amplitudes were obtained by digital substraction of inward current recorded in a 10^{-10} mol/l TTX solution of a given pH value from the current in TTX-free solution of the corresponding pH. Moreover, in TTX-free solutions the amplitude of the total inward current increased and its kinetics changed when the cell was transferred from a solution with pH 7.4 to a solution with pH 6.8 or pH 8.2. No similar changes were observed if 10^{-10} mol/l tetrodotoxin-containing solutions or chloride-free solutions were used. It can be supposed that changes in external pH affect the same mechanism which is responsible for changes in the total inward current in the presence of low TTX concentrations.

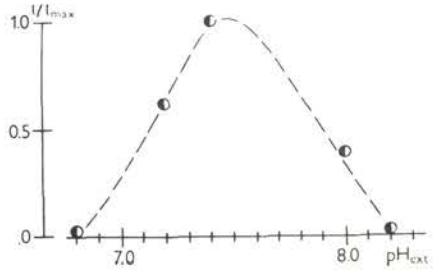

Fig. 11. The dependence of the difference current on external pH (normalized). The current in 10^{-10} mol/l TTX-containing solution was digitally subtracted from the initial trace for each external pH value. Abscissa: external values, linear scale. Experiment on a single rat ventricular myocyte. $V_h = -120$ mV; $V_t = -20$ mV; intracellular solution 1.

Discussion

Possible ionic nature of the difference current

One way to explain changes in inward current parameters due to the action of low external TTX concentrations is to postulate the existence of a tetrodotoxin-sensitive outward current with kinetic properties close to those of inward currents. In this case, blocking of the outward current (which is superimposed on inward currents under normal conditions) should induce an increase of the inward current amplitude. An alternative explanation suggesting induction of extra inward current channels by TTX, or modulation of the permeability of the existing inward current channels, seems much less probable. Experiments with changes in $[Na^+]_o$ are strongly conclusive (Fig. 7): the amplitude of the inward current increased when the cardiomyocyte was transferred from a solution with normal external sodium concentration to a solution with a lowered Na^+ concentration. At the presence of 10^{-10} mol/l TTX in external solution, or with Cl^--free solutions, a decrease in $[Na^+]_o$ induced a corresponding decrease of the inward current amplitude. This observation can be explained only by the abolishment of the compensating outward current by low Na^+ or TTX.

What is the ionic nature of such a peculiar current? In our experimental conditions, any pronounced cationic outward current was eliminated. The outward (in terms of voltage clamp) current could therefore be carried by chloride ions entering the cell.

The existence of different kinds of chloride permeability has been shown on a number of excitable objects: *Xenopus* oocytes (Miledi 1982; Barish 1983; Miledi and Parker 1984); *Aplysia* neurones (Chesnoy-Marchais 1983); mammalian spinal ganglia neurons (Owen et al. 1984; Mayer 1985).

First suggestions concerning the existence of a transient anion permeability in the membrane of heart muscle cells were made simultaneously by Carmeliet (1961a, b) and Hutter and Noble (1961). Their conclusions were based on changes in action potential parameters and in the spontaneous activity of Purkinje fibers as a result of the substitution of different anions for extracellular chloride. An outward current of nonidentified ionic nature was observed in the membrane of Purkinje fibers under voltage clamp conditions (Deck et al. 1964; Deck and Trautwein 1964). A strong reduction of this current in solutions with nonpermeable anions substituted for Cl^- allowed to define it as a chloride current (Dudel et al. 1967). This outward current, termed I_{to} (transient outward current), or "positive dynamic current" (Peper and Trautwein 1968), or "initial outward current" (Reuter 1968), was studied during the seventies (Vitek and Trautwein 1971; Fozzard and Hiraoka 1973; Hiraoka and Hiraoka 1973, 1975; Gibbons and Fozzard 1975). The current called I_{qr} (Cl^- as a possible carrier) was used as a fast repolarizing current in the model of electrical activity of Purkinje fibers (McAllister et al. 1975).

Later, Kenyon and Gibbons (1977) have shown that the substitution of different anions for chloride resulted in changes in Ca^{2+} activity in external solutions. When Ca^{2+} ions activity in external solutions was maintained constant, the elimination of Cl^- ions from the extracellular medium diminished the peak value of I_{to} to a much lesser extent (Kenyon and Gibbons 1979a). It was also shown that I_{to} could be blocked by potassium channel blokers, tetraethylammonium (TEA) and 4-aminopyridine (Isenberg 1978; Kenyon and Gibbons 1979a, b). I_{to} has therefore been suggested to be a potassium current. It was also shown that Cs^+ ions (K^+ channel blockers) introduced inside the cell suppressed I_{to} (Marban and Tsien 1982). At the same time, I_{to} could be blocked by Ca^{2+} antagonist D-600, and was reduced when Sr^{2+} or Ba^{2+} ions were substituted for extracellular calcium (Siegelbaum et al. 1977). These findings led to the suggestion of Ca^{2+}-dependence of this current. Stabilization of intracellular calcium by EGTA resulted in the disappearence of I_{to} (Siegelbaum and Tsien 1980). Later, Boyett (1981) and Coraboeuf and Carmeliet (1982) suggested that I_{to} could be a sum of two outward potassium currents with different kinetic properties and sensitivities to Ca^{2+} antagonists.

On the other hand, Coronado and Latorre (1982) have shown that one of the channel types in the sarcolemma of cardiomyocytes may represent an electrically gated chloride channel, though the corresponding "macro"-current has not been observed. Based on our experimental data discussed in the present paper we can suggest that this chloride "macro"-current was separated by TTX in isolated heart muscle cells.

The sodium dependence of the TTX-sensitive difference current described in the present paper can explain the lack of any literary data on such a perculiar TTX-effect in cardiomyocytes. Hitherto experimental investigations of tetrodotoxin action on membrane permeability of myocardial cells have almost exclusively been carried out in low-sodium or chloride-free solutions (Cohen et al. 1981; Brown et al. 1981). Also low TTX-effect could not be observed by other investigators owing to more positive (as compared with -120 mV in our investigations) holding potentials; due to a strong steady-state inactivation of the tetrodotoxin-sensitive difference current, it is already absent at holding potentials more positive than -70 mV.

It is worth mentioning that the observed TTX-sensitive "difference current" (see also Verkhratsky and Pidoplichko 1985) showed a steep dependence on pH of the external solution, and disappeared completely at pH_0 values lower than 6.8 or higher than 8.2. It is known that a shift in external pH can induce cardiac arrhythmias. The high sensitivity of the discovered current component to even slight acidification or alkalinization allows a tentative suggestion concerning the participation of this component in the response of the cardiac muscle to such perturbations.

The results of our experiments cannot be considered as bringing conclusive evidence for the existence of a transient TTX-sensitive chloride current carried through specific voltage gated chloride channels in the membrane of isolated cardiomyocytes. Nevertheless, at present state of our understanding of the phenomenon, this suggestion seems to be the least complicated way how to explain our data. Final conclusions about the nature, properties and physiological significance of the low-TTX-effect observed cannot be drawn until more information has been available.

References

Barish M. (1983): A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. J. Physiol. (London) **342**, 309—325

Boyett M. R. (1981): Two transient outward currents in cardiac Purkinje fibres. J. Physiol. (London) **320**, 32P

Brown A. M., Lee K. S., Powell T. (1981): Sodium current in single rat heart muscle cells. J. Physiol. (London) **318**, 479—500

Carmeliet E. (1961a): Chloride ions and the membrane potential of Purkinje fibres. J. Physiol. (London) **156,** 375–388

Carmeliet E. (1961b): Chloride and Potassium Permeability in Cardiac Purkinje Fibres. Arscia S.A., Brusels

Chesnoy-Marchais D. (1983): Characterization of a chloride conductance activated by hyperpolarization on *Aplysia* neurones. J. Physiol. (London) **342,** 277–308

Cohen Ch. J., Bean B. P., Colatsky T. J., Tsien R. W. (1981): Tetrodotoxin block of sodium channels in rabbit Purkinje fibers. Interaction between toxin binding and channel gating. J. Gen. Physiol. **78,** 383–411

Coraboeuf E., Carmeliet E. (1982): Existence of two transient outward currents in sheep cardiac Purkinje fibers. Pflügers Arch. **392,** 352–359

Coronado R., Latorre R. (1982): Detection of K^+ and Cl^- channels from calf sarcolemma in planar lipid bilayer membranes. Nature (London) **298,** 849–852

Deck K. A., Trautwein W. (1964): Ionic currents in cardiac excitation. Pflügers Arch. **280,** 63–80

Deck K. A., Kern R., Trautwein W. (1964): Voltage clamp technique in mammalian cardiac fibres. Pflügers Arch. **280,** 50–62

Dudel J., Peper K., Rüdel R., Trautwein W. (1967): The dynamic chloride component of membrane current in Purkinje fibers. Pflügers Arch. **295,** 197–212

Fozzard H. A., Hiraoka M. (1973): The positive dynamic current and its inactivation properties in cardiac Purkinje fibres. J. Physiol. (London) **234,** 569–586

Fozzard H. A., January C. T., Makielski J. C. (1985): New studies of the excitatory sodium currents in heart muscle. Circ. Res. **56,** 475–485

Gibbons W. R., Fozzard H. A. (1975): Slow inward current and contraction of sheep cardiac Purkinje fibers. J. Gen. Physiol. **65,** 483–510

Hiraoka M., Hiraoka M. (1973): Effect of low temperature on the positive dynamic current of cardiac Purkinje fibres. J. Physiol. (London) **234,** 587–595

Hiraoka M., Hiraoka M. (1975): The role of the positive dynamic current on the action potential of cardiac Purkinje fibers. Jpn. J. Physiol. **25,** 705–717

Horackova M., Vassort G. (1982): Effects of chloride replacement in cardiac muscle: mechanism of positive inotropy. J. Mol. Cell. Cardiol. **14,** 123–130

Hutter O. F., Noble D. (1961): Anion conductance of cardiac muscle. J. Physiol. (London) **157,** 335–350

Irisawa H. (1984): Electrophysiology of single cardiac cells. Jpn. J. Physiol. **34,** 375–388

Isenberg G. (1978): The positive dynamic current of the cardiac Purkinje fiber is not a chloride but a potassium current. Pflügers Arch. **377,** R5

Kenyon J. L., Gibbons W. R. (1977): Effects of low chloride solutions on action potentials of sheep cardiac Purkinje fibers. J. Gen. Physiol. **70,** 635–660

Kenyon J. L., Gibbons W. R. (1979a): Influence of chloride, potassium and tetraethylammonium on the early outward current of sheep cardiac Purkinje fibers. J. Gen. Physiol. **73,** 117–138

Kenyon J. L., Gibbons W. R. (1979b): 4-amynopiridine and the early outward current of sheep cardiac Purkinje fibers. J. Gen. Physiol. **73,** 139–157

Kostyuk P. G., Krishtal O. A., Pidoplichko V. I. (1981): Intracellular perfusion. J. Neurosci. Meth. **4,** 201–210

Marban E., Tsien R. W. (1982): Effects of nystatin-mediated intracellular ion substitution on membrane currents in calf Purkinje fibers. J. Physiol. (London) **329,** 569–587

Mayer M. L. (1985): Calcium-activated chloride current in rat dorsal root ganglion neurones. J. Physiol. (London) **361,** 22P

McAllister R. E., Noble D., Tsien R. W. (1975): Reconstruction of the electrical activity of cardiac Purkinje fibres. J. Physiol. (London) **251,** 1–59

Miledi R. (1982): A calcium-dependent transient outward current in *Xenopus laevis* oocytes. Proc. Roy. Soc. London **B215,** 491—497

Miledi R., Parker J. (1984): Chloride current induced by injection of calcium into *Xenopus* oocytes. J. Physiol. (London) **357,** 173—183

Owen D. G., Barker J. L., Segal M. (1984): A Ca^{2+}-dependent Cl^- conductance in cultured mouse spinal neurones. Nature (London) **311,** 567—570

Peper K., Trautwein W. (1968): A membrane current related to the plateau of the action potential of Purkinje fibers. Pflügers Arch. **303,** 108—123

Pidoplichko V. I. (1983): The technique of "step-by-step" and "rectangular" hydrodynamic application of substances to the membrane of perfused neurones. Fiziol. Zh. **29,** 754—757 (in Russian)

Pidoplichko V. I. (1986): Two TTX-separable sodium inward currents in the membrane of isolated cardiomyocytes. Gen. Physiol. Biophys. **6,** 593—604

Reuter H. (1968): Slow inactivation of currents in cardiac Purkinje fibers. J. Physiol. (London) **197,** 233—253

Reuter H. (1984): Ion channels in cardiac cell membranes. Annu. Rev. Physiol. **46,** 473—484

Siegelbaum S. A., Tsien R. W. (1980): Calcium activated transient outward current in calf cardiac Purkinje fibers. J. Physiol. (London) **299,** 485—506

Siegelbaum S. A., Tsien R. W., Kass R. S. (1977): Role of intracellular calcium in the transient outward current in calf Purkinje fibres. Nature (London) **269,** 611—613

Verkhratsky A. N., Pidoplichko V. I. (1985): Tetrodotoxin-sensitive pH-dependent chloride current in the membrane of isolated cardiomyocytes. Biol. Membrany **2,** 17—24 (in Russian)

Vitek M., Trautwein W. (1971): Slow inward current and action potential in cardiac Purkinje fibres. Pflügers Arch. **323,** 204—218

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