Membrane Current Changes Induced by Acetylstrophanthidin in Cardiac Purkinje Fibers

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SUMMARY

Voltage clamp experiments were carried out with short Purkinje fibers exposed to acetylstrophanthidin. A consistent change in membrane current was an appearance of a transient inward current on repolarization to the resting potential from the preceding depolarization, when preparations were treated with sufficient concentration of acetylstrophanthidin to cause the transient depolarization. This transient inward current displayed voltage- and time-dependence on the preceding depolarization, of which kinetics were different from the pacemaker K⁺ current. The transient inward current was easily blocked by manganese ions. The results indicated that acetylstrophanthidin induced the transient inward current which was the basis of the transient depolarization and the current might represent an abnormal state of the slow inward current possibly related to the altered internal Na⁺ and Ca²⁺ concentrations.

Additional Indexing Words:

Digitalis intoxication Transient depolarization Abnormal automaticity Voltage clamp Slow inward current

CARDIAC glycosides are known to cause various arrhythmias as the manifestation of their toxic effects. One of the earliest signs of digitalis intoxication demonstrated in the microelectrode studies was recently shown to be a transient depolarization, which developed in Purkinje fibers but not in ordinary working myocardium. This transient depolarization (TD) appeared to be coupled with the preceding excitation and seemed not to represent a reentrant activity. It is also assumed that TD probably represents abnormal automaticity different from the normal pacemaker mechanism. Membrane current underlying normal pacemaker activity is believed to be carried by a pacemaker potassium current (I_k). However, the mechanism underlying TD induced by digitalis has not been clarified yet, although the involvement of transmembrane Ca²⁺ current was suggested. The present experiments show an appearance and some properties of transient inward
current in short Purkinje fibers after exposure to acetylstrophanthidin (A. str.), which possessed different characteristics from the pacemaker K+ current.

**Methods**

All the experiments were carried out with free-running Purkinje strands dissected from the left ventricle of the young sheep. In the experiments recording action potentials, long (about 5–10 mm) Purkinje fibers were used. In the voltage clamp experiments, short (1–2 mm) Purkinje fibers were prepared. The technique of the voltage clamp employed for the present experiments was the double micro-electrodes method similar to that originally described by Deck et al. The technique and the method of preparations in the present experiments were same as the previous report and their detailed description was presented in the previous paper. The composition of the Tyrode solution in mmol/liter (mM/L) was: NaCl, 137; KCl, 5.4; CaCl₂, 1.8; MgCl₂, 0.5; NaHCO₃, 13.4; NaH₂PO₄, 2.4; glucose, 11.1. Manganese solution was made up by containing 5 mM MnCl₂ in 10 mM Tris buffer instead of bicarbonate buffer and other composition was same as the Tyrode solution. Acetylstrophanthidin (A.str.) was prepared as the final concentration of 2.5–5.0×10⁻⁷ M/L dissolved in the Tyrode solution. The pH of the solution was adjusted to 7.2–7.4. All the solutions were equilibrated with 95% O₂-5% CO₂ except Mn⁺⁺ solution which was aerated with 100% O₂ throughout the experiments. The temperature of the bath was maintained at 36–37°C.

**Results**

(1) The effects of acetylstrophanthidin on Purkinje action potentials:

When long Purkinje fibers were exposed to 2.5×10⁻⁷ M/L of A. str. for more than 30 min, the following changes of action potentials were always observed: The resting membrane potential was depolarized by an average of 13.6 mV±3.42 (S.E.) in 4 fibers. The size of action potentials and the overshoot were decreased. The upstroke velocity of action potentials was also diminished. The plateau and the duration of action potentials became shorter than the control. In addition to these changes, a low amplitude depolarization following each action potential was observed after exposure to A. str. for 20–45 min in all 4 fibers studied. Fig. 1 demonstrates one of the example which showed the development of this low amplitude depolarization after exposure to A. str. and its disappearance after 60 min of wash-out of the drug. This low amplitude depolarization resembled the TD reported by Ferrier et al1). Therefore the author also used the term TD for this depolarization. The TD reached its peak at 300–500 msec after the end of repolarization of the previous excitation with the basic cycle length of 1,700 msec and occasionally it reached the threshold to develop the extra excitations as already described.
In each picture, the top horizontal line represents 0 mV of the membrane potential and dV/dt of the upstroke of action potential. (A) is the control recording. (B) is the record taken after exposure to A. str. for 30 min. Note the development of the TD after repolarization of action potential as indicated by a white arrow. The slope of slow diastolic depolarization became less steep than in the control. (C) is the record taken 60 min after wash-out of the drug and there is no TD.

by Ferrier et al. The slope of slow diastolic depolarization became less steep during the development of TD than in the control.

(2) The effects of acetylstrophanthidin on membrane current in short Purkinje fibers:

To study the mechanism underlying this TD, the voltage clamp experiments were carried out with short (less than 2 mm) Purkinje fibers before and after exposure to A. str. When short Purkinje fibers were superfused with 2.5–5.0×10⁻⁷ M/L of A. str., the most prominent and consistent change in the membrane currents was an appearance of a transient inward current following repolarization to the resting (holding) potential after the depolarizing pulses. Fig. 2 demonstrates the voltage clamp records taken from the same
Fig. 2. Voltage clamp records in the short Purkinje fiber before (A) and after (B) exposure to A.str. In each picture, the upper beam represents membrane current and the lower beam shows membrane voltage. Transient inward current as indicated by white arrows appeared after repolarization to holding potential from depolarizing pulses of 800 msec duration in (B). Note the larger size and the earlier appearance of the transient inward current with increasing depolarization of the preceding pulses.

Fig. 3. Dependence of transient inward current on preceding depolarization. The abscissa represents the level of preceding depolarization (V<sub>I</sub>). The ordinate indicates the amplitude (I) of transient inward current (upper half) and the time to peak (t) of the current after repolarization (lower half). The measurement of two parameters (I and t) are schematically shown in the inset.
fiber before and after 30 min exposure to $5 \times 10^{-7} \text{M/L}$ of A. str. When the membrane potential was repolarized from depolarizing square pulses with 800 msec duration to the resting potential ($-75 \text{ mV}$ in the case of Fig. 2), a transient inward current with rather slow time course was observed after A. str. (B) as indicated by white arrows, which was never seen in the control recordings (A). This transient inward current increased its amplitude and its appearance became earlier after repolarization with larger preceding depolarization. Fig. 3 shows the analysis of these changes of the current in the similar experiment but obtained from another fiber immersed in $2.5 \times 10^{-7} \text{ M/L}$ of A. str. for 30 min. In this figure, the ordinate represents the amplitude of the transient inward current and its time to peak from the point of repolarization, and the abscissa shows the level (membrane potential) of the preceding depolarization. The results clearly demonstrated the dependence of this transient inward current on the level of the preceding depolarization. Same type of changes as shown in Figs. 2 and 3 were always observed in 4 fibers examined. The time to peak of this current after repolarization was varied not only with the level of the preceding depolarization but also the concentrations of A. str. used and different fibers. But generally the values fell in the range between 150 to 600 msec with 800 msec depolarizing pulses applied at the frequency of 0.25 Hz.

In addition to an appearance of the transient inward current, the cur-

![Graph](image)

**Fig. 4.** Dependence of transient inward current on the duration of preceding depolarization.

With increasing pulse duration, the amplitude of transient inward current becomes larger but its mode of increase does not fit a single exponential curve.
rent records in Fig. 2 seem to show some increase in slow inward current after A. str. However, this was not a consistent change in the present experiments. Three other fibers did not show any increase in the slow inward current at all, although they all showed transient inward current on repolarization. Thus the appearance of the transient inward current was not necessarily paralleled with concomitant increase in the activation of the slow inward current.

The effect of the duration of the preceding depolarization on this inward current was also examined and one of the results is shown in Fig. 4. The result indicated the dependence of the current amplitude and time to peak on the pulse duration of preceding depolarization. The current amplitude increased when the pulse duration was prolonged and it decreased at the short pulse. The mode of increase was not a single exponential fashion with increasing duration but it showed some sigmoid dependence on the duration of the preceding depolarization.

![Fig. 5. Voltage clamp records showing the effect of Mn++ on transient inward current.](image)

(A) Control
(B) A·str. for 30 min.
(C) 5mM-Mn++ for 5 min.

Fig. 5. Voltage clamp records showing the effect of Mn++ on transient inward current.

(A) is the control record. (B) is the record taken 30 min after exposure to A·str. Transient inward current appeared as indicated by a white arrow. (C) is the record taken 5 min after perfusion of 5 mM-Mn++ Tyrode solution. The transient inward current disappeared.
Finally the effects of manganese ions were tested on this transient inward current, since the appearance of TD was implicated by the inflow of Ca++ current and Mn++ was shown to be a specific blocker of this current. As can be seen in Fig. 5, 5 mM-Mn++ quickly abolished the transient inward current within a short period of perfusion (5 min).

DISCUSSION

When A. str. was applied to Purkinje fibers with its sufficient concentration to induce arrhythmias, the consistent change in the membrane current after drug application was a development of a transient inward current after repolarization from the preceding depolarization. The current showed rather slow time course reaching its peak at 150–600 msec after the end of repolarization.

The current is not likely to represent an artifact because of the following reasons; in such a small preparation (less than 2 mm in length), the timing of the current appearance was too slow to be attributed to the conducted action potential under the condition of non-homogeneous voltage control. From our previous experiments, this method was shown to achieve good voltage control throughout the preparation except first 10 msec after the voltage change where membrane conductance fell considerably. But the timing of the transient inward current was far outside of this critical range. Finally this current was never observed in the non-treated fibers and was only seen after an application of the drug. Therefore it can reasonably be stated that the transient inward current is a true membrane current change induced by A. str.

The transient inward current was only seen after repolarization from the preceding depolarization and it showed clear dependence on the level and the duration of depolarizing pulses. These characteristics were quite compatible with the natures of TD reported by previous authors; namely, TD became apparent after stopping the train of impulses or after each action potential. The slope and the magnitude of TD became steeper and larger with the shorter basic cycle length of the stimulation. Furthermore TD was abolished by manganese ions. Therefore it might be safe to argue that the transient inward current observed in the voltage clamp experiments was the basis of TD observed in the recordings of membrane potentials.

The appearance of the transient inward current in Purkinje fibers after exposure to A. str. was recently reported by Lederer and Tsien in a preliminary form, and their findings were similar to the present observations. However the present experiments further demonstrated the dependence of
the current on the level and the duration of the preceding depolarization. The voltage dependence of this current was different from the property of the normal pacemaker current \((iK_2)\)^4 showing increased current amplitude at the voltage more positive to \(-60\) mV. It was also shown that ouabain decreased the magnitude of \(I_k2\) of Purkinje fibers.\(^{11}\) Moreover, the phase 4 depolarization which was believed to be caused by \(I_k2\) was depressed during the development of TD\(^1\) (and Fig. 1 of this paper). These results were consistent with the idea that the TD was not brought about by the change in \(I_k2\).

The sort of time and voltage dependence of this current was quite different from the kinetic behaviors of any other currents reported so far.\(^{12,13}\) The current was also shown to be blocked by manganese ions which possessed the ability to inhibit \(Ca^{++}\) current.\(^8\) However manganese sensitivity itself appeared not to support the idea that this current represented a normal property of the slow inward current, since membrane currents in response to depolarizing pulses did not show consistent increase in the slow inward current. Alternatively, it may represent a modified nature of the slow inward current in its inactivation process, somehow related to \(Ca^{++}\) and/or \(Na^{+}\) movements in the cytoplasm, or it may represent a different current system induced by the drug. The precise understanding as to the basic mechanism of this current awaits further experimental analysis.

**Addendum:** After the submission of this paper, an article by Lederer WJ and Tsien RW (J Physiol 236: 73, 1976) appeared, which described the detail about the nature of this transient inward current. The present results essentially agreed with their findings.

**REFERENCES**

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