Action of Dantrolene Sodium on Electrical and Mechanical Activity of Guinea-pig Ventricular Muscles

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Abstract Effects of dantrolene sodium on electrical and mechanical activity of guinea-pig ventricular muscles were examined. The application of $2 \times 10^{-5}$ M of dantrolene caused a decrease of developed tension by 31% of the control level without noticeable changes in resting membrane potential, amplitude and maximum upstroke velocity ($V_{\text{max}}$) of action potentials, and action potential duration. Negative inotropic effects of dantrolene appeared after 10 min application and reached almost a plateau after 15–20 min. Neither amplitude of the slow inward current nor the outward current on depolarization were affected by dantrolene. When muscle preparations were superfused with the low-$K^+$, high-$Ca^{2+}$ solution, they developed delayed afterdepolarizations and aftercontractions. The application of $2 \times 10^{-5}$ M of dantrolene did not reduce amplitudes of both delayed afterdepolarizations and aftercontractions, but depressed twitch tension to 74% of the control level. Dantrolene did not change the amplitude of the transient inward current observed under the low-$K^+$, high-$Ca^{2+}$ condition. These results suggest that a primary site of action of dantrolene is not the sarcolemma of ventricular myocardium, but possibly the $Ca^{2+}$ releasing process from the sarcoplasmic reticulum. Its action is further indicated to affect the process of depolarization-induced $Ca^{2+}$-release, either directly or indirectly rather than to inhibit $Ca^{2+}$-induced $Ca^{2+}$-release.

Key words: dantrolene-$Na^+$, negative inotropic effect, slow inward current, transient inward current, $Ca^{2+}$-release.

Dantrolene sodium is widely known as a skeletal muscle relaxant and used for the treatment of various forms of muscle spasticity (Pinder et al., 1977). Recently, the drug has also been employed effectively in the prevention and treatment of malignant hyperthermia syndrome, which has various manifestations in the cardiovascular system including life-threatening arrhythmias, in addition to the pyrexic
crisis (Britt, 1979; Huckell et al., 1978). The latter point may indicate that the drug affects the electrical activity of heart muscles. The effects of dantrolene sodium (dantrolene) on mechanical and electrical properties of cardiac muscle, however, are controversial; some authors described a positive inotropic action associated with a possible increase in the slow inward current as its primary effect (Hatae et al., 1980; Mézárós et al., 1981), while others reported opposite or biphasic actions with or without changes in slow action potentials (Mézárós et al., 1982; Salata and Jalife, 1982; Salata et al., 1983). The former results can possibly be explained by the later observations (Honérjäger and Alischewski, 1983; Mézárós et al., 1982) that the drug caused catecholamine release from nerve endings distributed in the myocardium. Further, Honérjäger and Alischewski (1983) suggested that dantrolene had no influence on the slow inward current as judged by its effect on slow action potential. However, direct proof of this view is still lacking, since the measurement of the membrane current under the influence of dantrolene has not been undertaken so far, and the slow action potential is influenced by changes in other current systems in addition to the slow inward current (Reuter, 1979). The study of Honérjäger and Alischewski (1983) also implicated a possible site of the drug action to be the Ca\(^{2+}\)-releasing process from the sarcoplasmic reticulum. However, it has not yet been established which of the several Ca\(^{2+}\)-releasing processes are affected by dantrolene.

Recently, the development of the delayed afterdepolarizations (DADS) has been observed in many cardiac tissues under various experimental conditions (Cranefield, 1975; Ferrier, 1977). We have also reported their appearances in mammalian ventricular muscle fibers during exposure to the low-K\(^+\), high-Ca\(^{2+}\) solution (Hiraoka et al., 1979, 1981a). The mechanism of origin of the DADS has been suggested to be due to the cyclic release of Ca\(^{2+}\) from the sarcoplasmic reticulum under the conditions of the Ca\(^{2+}\)-overload in the cell (Kass et al., 1978).

The present study was done to examine actions of dantrolene on electrical properties, including membrane current changes of guinea-pig ventricular muscle fibers, when the drug shows inotropic effects. The drug action was also tested with preparations demonstrating DADS, associated tension (aftercontraction), and the underlying current (transient inward current) under the low-K\(^+\), high-Ca\(^{2+}\) condition in order to clarify its possible intracellular site of action. A preliminary report of this work has appeared elsewhere (Kinoshita et al., 1983).

**METHODS**

Guinea-pigs weighing 250–350 g were stunned with a blow on the neck, and their hearts were rapidly removed through a thoracotomy. Papillary muscles were dissected from the right ventricle.

*Recordings of the membrane potential and the isometric tension.* The usual size of these preparations used in both potential and tension measurements was
2-4 mm in length and 0.8-1.5 mm in diameter. The preparations were placed in
the tissue chamber and superfused with a modified Tyrode's solution. The volume
of the tissue chamber was about 1 ml and the superfusate flow rate was set at 5.0-
5.5 ml/min using a constant perfusion pump (Tokyo Rikakikai Co., MP-3). The
composition of the Tyrode's solution was (mm/l); 125 NaCl, 4.0 KCl, 1.8 CaCl₂,
1.0 MgCl₂, 0.4 NaH₂PO₄, 25.0 NaHCO₃, and 5.5 glucose. The low-K⁺, high-Ca²⁺
solution had 0 or 1 KCl and 3.6 CaCl₂, and other compositions were the same as the
Tyrode's solution. Dantrolene sodium (dantrolene) was dissolved first in me-
thanol and then added to the Tyrode's or the low-K⁺, high-Ca²⁺ solution to achieve
the final concentration of 2 x 10⁻⁵ M. Thus, the dantrolene solution contained
0.1% of methanol, which was shown to have no inotropic effect during 20 min
of the application to our preparations (3 fibers). We used a single dose of 2 x
10⁻⁵ M dantrolene, since the maximal solubility of the drug in physiological solu-
tions was less than 15 mg/l (=3.75 x 10⁻⁵ M) (ELLIS and CARPENTER, 1972), and
concentrations higher than this level might have had additional effects, depending
on solvents used. The pH of these solutions was 7.2-7.4. They were gassed with
95% O₂+5% CO₂ throughout the experiments. Temperature of the superfusate
was maintained at 36-37°C.

Glass microelectrodes filled with 3 M KCl, having resistances of 10-20 MΩ
and tip potentials less than 5 mV, were used to record membrane potentials. The
microelectrode was coupled with an Ag-AgCl electrode and connected to the input
stage of high-impedance capacitance neutralizing amplifier (WPI, model 750). The
maximum upstroke velocity (Vₘₐₓ) of the action potential was obtained using
an electronic differentiation as described elsewhere (HIRAOKA et al., 1981a). Iso-
metric tension was measured using a mechano-electric transducer (ME Commercial
Co., ME-4021). The transducer was mounted on a micromanipulator which al-
lowed a controlled stretching of the preparation to a length at which developed
tension was about 90% of the maximum. Records of membrane potentials and
tension were displayed on an oscilloscope (Nihon Kohden Co., VC-10) and photo-
graphed with a camera (Nihon Kohden Co., PC-2B) or inscribed with a direct
writing recorder (San-ei Sokki Co., Rectigraph 8S). Preparations were stimulated
using rectangular pulses (2 msec in duration and twice the threshold voltage) ob-
tained from a pulse generator (Nihon Kohden Co., SEN-7103). Stimuli, passed
through an isolation transformer, were delivered to one end of the preparations
through bipolar silver electrodes.

The preparations were stabilized in a tissue chamber superfused with the
Tyrode's solution for 60 min and stimulated with a frequency of 1 Hz. At the end
of this stabilizing period, shapes of action potentials and of developed tensions were
carefully watched for another 20 min. After we recorded stable configurations of
action potentials and developed tensions during the observation period, a 2 x
10⁻⁵ M dantrolene was applied to muscle preparations during 20 min followed by a
washout period of 60-80 min. In three preparations, the application of dantrolene
was prolonged to 60 min (see RESULTS). The preparations in which we could maintain a stable impalement of the electrode in the same cell during the application of the drug were selected as a data analysis.

Inductions of the delayed afterdepolarizations (DADs) and the aftercontractions (ACs) were done as follows: after stabilization in the Tyrode’s solution, the preparations were superfused with the low-K\(^+\), high-Ca\(^{2+}\) (1 mM-K\(^+\), 3.6 mM-Ca\(^{2+}\)) solution for 30 min. They developed DADs and the ACs following the train of ten applied impulses with frequencies of 1-3.3 Hz. Some preparations showed triggered-activity following the train and they were discarded from the further experiments. If the preparations constantly responded to repeated applications of the train impulses in the development of the DADs and the ACs during the 20 min of the observation period, a \(2 \times 10^{-5}\) M dantrolene was then applied to the preparation during 20 min followed by the wash-out period.

Voltage clamp experiments. Membrane current changes were examined using a single sucrose gap voltage clamp technique. The descriptions of our single sucrose gap method and the voltage clamp apparatus appeared in the previous communication (HIRAOKA et al., 1981b). The membrane voltage was monitored by the microelectrode technique described above. After the membrane currents, in response to depolarizing pulses of 1 sec duration given every 5 sec, were measured in the Tyrode’s solution, a \(2 \times 10^{-5}\) M of dantrolene was applied to the test compartment for 20 min and the same protocol of the voltage clamping was repeated. To induce the transient inward current, the test compartment was superfused with the low-K\(^+\), high-Ca\(^{2+}\) solution (0 mM-K\(^+\), 3.6 mM-Ca\(^{2+}\)) (see EISNER and LEDERER, 1979). The current on repolarization from the depolarizing pulses of 1 sec duration was easily observed in this solution after 20 min. After we obtained a stable appearance of the transient inward current in response to the above clamp protocol during the 20 min of the observation period under this condition, the dantrolene in the low-K\(^+\), high-Ca\(^{2+}\) solution was applied for 20 min and its effect on the transient inward current was examined.

Comparisons were performed by a paired t-test. Differences were considered to be significant only when \(p\) values were less than 0.05. All data are expressed as the mean±S.E.M.

RESULTS

Effects of dantrolene on electrical and mechanical activities in the Tyrode’s solution

While the membrane potential and the twitch tension were simultaneously recorded in the Tyrode’s solution, effects of dantrolene in both parameters were examined using seven preparations. The application of \(2 \times 10^{-5}\) M of dantrolene caused a mild depression of the twitch tension without any noticeable changes in the membrane potential (Fig. 1). The effect on the tension started to develop after 2 min of the drug application and reached a near maximal level after 3 min in this
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This fiber responded fastest to the drug. The average time of the appearance of the drug effect was 7 min of the application, and the maximal level was achieved at about 14 min in seven preparations. During the wash-out period of the drug after 20 min of application, the tension slowly recovered to the control level in 25–60 min. Figure 2 demonstrates the time course of the dantrolene effects on the action potential duration (APD) and the twitch tension. The effects on the APD were tested at three different levels. Dantrolene did not affect 20, 50, and 90% APD. In three additional fibers, dantrolene was applied for 60 min and the suppression of the twitch was shown to stay at about the same level after 20 min of the treatment.

Table 1 shows a summary of the effects of $2 \times 10^{-5}$ M of dantrolene during 20 min of treatment on electrical and mechanical parameters. Dantrolene produced no changes in the electrical parameters, including the resting membrane potential, the amplitude and the $V_{\text{max}}$ of the action potential, and three different levels of APDs. The drug reduced the twitch amplitude by 31% of the control level and abbreviated the time to peak of the twitch tension by about 9% of the control. The relaxation time was prolonged by 10% during the drug application.

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Fig. 1. Effects of dantrolene on the action potential and the twitch tension. The upper row represents pictures taken from the oscilloscopic screen. In each picture, the top line of the right corner indicates the $V_{\text{max}}$ of the action potential, the middle line shows the action potential, and the bottom is the tension. The lower row shows the chart recordings of the membrane potential (upper record; $E_m$) and the tension (lower record; $T$). Arrows indicate the time of recordings of the pictures shown at the upper row. The application of dantrolene caused a mild depression of the twitch tension (B) followed by its recovery during the wash-out period of the drug (C), and without any changes in the membrane potential.
The effect of dantrolene on the slow inward current was examined using a voltage clamp technique. Depolarizing clamp pulses to various voltages with 1 sec duration were applied to the preparations from the holding potential which was held at about −40 mV so as to inactivate the fast Na⁺ current. The changes in the membrane current in response to depolarizing clamps were examined before and during the application of $2 \times 10^{-5}$ M of dantrolene, and at 20 min after the superfusion with the drug-free solution. Figure 3 shows a typical example of the experimental records on the left (I) and the analyses of the current-voltage relationship of the steady-state current and of the slow inward current on the right (II).

Fig. 2. Time-course of changes in the APD and the tension while applying dantrolene. The abscissa indicates the time. Dantrolene was applied from 0 to 20 min as indicated by the box. The ordinate shows the APD in msec at the top and the relative tension in % at the bottom. The APD was measured at three different levels. In each preparation, the relative tension was expressed in the value of the twitch amplitude before applying dantrolene as 100%. Preparations were stimulated with the frequency of 1 Hz throughout the experiments. Each symbol with bars indicates the mean value ± M.S.E. from seven experiments. Abbreviations APD₈₀, 90% APD; APD₅₀, 50% APD; APD₂₀, 20% APD; Dant, dantrolene; WO, wash-out of dantrolene. ** p<0.05. The p values were obtained from comparisons between the value before dantrolene and that at each measurement time.

The effect of dantrolene on the slow inward current was examined using a voltage clamp technique. Depolarizing clamp pulses to various voltages with 1 sec duration were applied to the preparations from the holding potential which was held at about −40 mV so as to inactivate the fast Na⁺ current. The changes in the membrane current in response to depolarizing clamps were examined before and during the application of $2 \times 10^{-5}$ M of dantrolene, and at 20 min after the superfusion with the drug-free solution. Figure 3 shows a typical example of the experimental records on the left (I) and the analyses of the current-voltage relationship of the steady-state current and of the slow inward current on the right (II).
Table 1. Effects of dantrolene on electrical and mechanical parameters of guinea-pig ventricular muscle fibers. Data were collected from seven experiments stimulated with a frequency of 1 Hz. $2 \times 10^{-5}$ M dantrolene was applied to the preparations for 20 min and the results obtained at the end of the dantrolene superfusion were collected. The values on the wash-out of the drug were obtained after 30–60 min superfusion by the drug-free solution.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before dantrolene</th>
<th>Dantrolene</th>
<th>Wash-out dantrolene</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP (mV)</td>
<td>$-95.5 \pm 1.7$</td>
<td>$-95.7 \pm 1.6$</td>
<td>$-95.4 \pm 1.5$</td>
</tr>
<tr>
<td>APA (mV)</td>
<td>$127.9 \pm 1.3$</td>
<td>$127.9 \pm 1.7$</td>
<td>$128.5 \pm 1.6$</td>
</tr>
<tr>
<td>$V_{\text{max}}$ (V/sec)</td>
<td>$249.2 \pm 21.9$</td>
<td>$232.6 \pm 17.7$</td>
<td>$241.2 \pm 19.9$</td>
</tr>
<tr>
<td>20% APD (msec)</td>
<td>$82.3 \pm 1.7$</td>
<td>$78.8 \pm 2.1$</td>
<td>$80.2 \pm 4.2$</td>
</tr>
<tr>
<td>50% APD (msec)</td>
<td>$148.3 \pm 3.6$</td>
<td>$155.0 \pm 4.2$</td>
<td>$153.4 \pm 5.6$</td>
</tr>
<tr>
<td>90% APD (msec)</td>
<td>$176.9 \pm 4.1$</td>
<td>$187.1 \pm 4.3$</td>
<td>$183.9 \pm 5.8$</td>
</tr>
<tr>
<td>Twitch (%)</td>
<td>100</td>
<td>$69.0 \pm 4.2^*$</td>
<td>$99.1 \pm 9.4$</td>
</tr>
<tr>
<td>Time-to-peak (msec)</td>
<td>$99.4 \pm 8.0$</td>
<td>$90.2 \pm 5.3*$</td>
<td>$95.6 \pm 6.4$</td>
</tr>
<tr>
<td>Relaxation-time (msec)</td>
<td>$153 \pm 6.8$</td>
<td>$168 \pm 4.3*$</td>
<td>$160 \pm 6.0$</td>
</tr>
</tbody>
</table>

* $p<0.05$, ** $p<0.01$. RMP: resting membrane potential, APA: action potential amplitude

Fig. 3. No effects of dantrolene on the membrane currents in the Tyrode's solution. The left (I) shows actual experimental records. In each picture, the upper line represents the membrane voltage ($V$) and the lower the membrane current ($I$). The holding potential was $-41$ mV and a depolarizing clamp of 1 sec duration to $+1$ mV was applied in each record. Neither the amplitude of the slow inward current nor that of the outward current at the end of the 1 sec pulse was affected while applying dantrolene (B). The right (II) shows the current-voltage relationship of the steady-state outward current and the slow inward current, obtained from the same experiments shown in the left (I).

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The amplitude of the steady-state current was measured by the difference between the holding current and the current at the end of the 1 sec pulse. The amplitude of the slow inward current was measured from the difference between the peak of the inward current and the current at the 200 msec of the pulse, as suggested by MCDONALD and TRAUTWEIN (1978). As can be seen, the application of dantrolene for 20 min did not affect the steady-state current and the slow inward current. Since the level of the holding current did not change appreciably among three stages of these experiments, steady-state outward current was not actually influenced by the drug. Similar results to those shown in Fig. 3 were confirmed in four preparations.

(A) Before Dantrolene
(B) 2 × 10⁻⁵ M Dantrolene 20min.
(C) Wash-out Dantrolene

Fig. 4. Effects of dantrolene on the membrane potential and the tension in the preparation developing the DADs and the ACs. In each picture, the top line represents the membrane potential, the middle line the tension, and the bottom the Vmax of the action potential. The preparation was given the train of 10 impulses and a frequency of 3 Hz at each trial. (A) shows the record obtained before the dantrolene application. The DADs were seen following each action potential. The twitch tension followed by the ACs showed a positive staircase phenomenon. (B) reveals the record obtained at 20 min using dantrolene. Although the amplitudes of the DADs and the ACs were barely affected, the twitch tension decreased with an elimination of the positive staircase during the train. (C) shows the obtained record on the wash-out of the drug. The amplitudes of the twitch and the positive staircase did not recover completely to the pre-dantrolene level.
Effects of dantrolene on electrical and mechanical activities in the low-K⁺, high-Ca²⁺ solution

When muscle preparations were superfused with the low-K⁺, high-Ca²⁺ solution, they developed delayed afterdepolarization (DAD) and aftercontraction (AC) following the train of applied impulses (EISNER and LEDERER, 1979; HIRAOKA et al., 1979, 1981a). Effects of 2 × 10⁻⁵ M of dantrolene on these activities and the last twitch evoked by the 10 impulses of the train were examined. Figure 4 shows one of the experimental records. When the train of 10 impulses with a frequency

![Fig. 5. Time-course of changes in the DAD, the AC, and the twitch tension using dantrolene. The abscissa indicates the time. Dantrolene was applied for 20 min as indicated by the box. The ordinate shows the amplitude of the DAD in mV at the top, the relative AC in % at the middle, and the relative twitch tension in % at the bottom. The results obtained were from similar experiments of seven preparations as shown in Fig. 4. All the preparations were given the train of 10 impulses of a frequency of 3 Hz. Measurements were done using the largest DAD and the AC following the last impulses, and with the tenth twitch for the tension. Both the relative AC and relative twitch were expressed the value before applying dantrolene as 100%. The amplitude of the DAD and the relative AC did not change during the application of the drug, while the relative twitch decreased after 10 min or later during the treatment and, then, showed an incomplete recovery during wash-out of dantrolene. * p <0.05, ** p <0.01.](image-url)
of 3 Hz was applied to the preparation, DADs and ACs were seen and twitch tension during the train showed a positive staircase phenomenon. Dantrolene did not cause any remarkable changes in amplitudes of both DADs and ACs, but reduced twitch amplitude and a positive staircase. The wash-out of the drug caused an incomplete recovery of the twitch and the positive staircase. Further changes in DADs and ACs were not observed during the wash-out period.

Figure 5 shows time course of changes in amplitudes of DAD and of AC, and the 10th twitch evoked by the train stimuli after applying dantrolene. The amplitudes of DAD and AC were not influenced significantly during the drug application, but twitch became depressed at 10 min or at a later time of application. Its effect was almost maximal at 15 min or later. After 20 min in dantrolene, the twitch became 74.0±7.5 (mean±M.S.E.) % of the control. It showed an incomplete recovery to the pre-dantrolene level during the wash-out period of the drug, since

![Diagram](image)

**Fig. 6.** Effect of dantrolene on the transient inward current. In each picture, the upper line represents the membrane voltage (V) and the lower is the membrane current (I). The holding potential was -41 mV and a depolarizing pulse of 1 sec duration to +1 mV was applied in each record. The transient inward current was observed following the repolarization to the holding potential from the preceding depolarization, before (A), during (B), and after (C) applying dantrolene.
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Lederer and Tsien (1976) have shown that the DADs in digitalis-inotoxicated Purkinje fibers are formed by transient inward currents (TIs). The presence of TIs in ventricular muscle fibers exposed to digitalis has also been demonstrated (Karaguezian and Katzung, 1982). In our preparations, we examined the effect of dantrolene on TIs. Figure 6 shows the voltage clamp records obtained before, during, and after the treatment of $2 \times 10^{-5} \text{M}$ of dantrolene. TI was seen following repolarization from preceding depolarization and dantrolene did not cause any change in terms of its amplitude and time-course. Similar results as those shown in Fig. 6 were confirmed in five preparations in the low-K+, high-Ca$^{2+}$ solution. Figure 7 demonstrates a relationship between activation voltage and TI, and the effect of dantrolene on it. As can be seen, dantrolene did not af-

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**Fig. 7.** Effects of dantrolene on the relationship between the activation voltage and the amplitude of the transient inward current. Experimental protocols for the measurement of the TI are shown in the inset. Depolarizing pulses of 1 sec duration to each membrane potential ($E_m$) were applied from the holding potential of $-42 \text{ mV}$, and this potential is plotted on the abscissa. The ordinate is the amplitude of the TI. Solid circles indicate values obtained before applying dantrolene, triangles are those during the treatment, and open circles those of the wash-out of the drug. Dantrolene did not affect the current-activation-voltage relationship of the TI.
fect the voltage dependency of activation of TIs at any applied membrane potentials examined, and this result was confirmed in five preparations.

DISCUSSION

The present experiments disclosed that dantrolene sodium produced a negative inotropic effect on the mammalian ventricular myocardium without any remarkable changes in electrical parameters, including the membrane current in the Tyrode's solution. The drug action on these normal cardiac preparations is similar to that observed in skeletal muscles (Ellis and Carpenter, 1972; Pinder et al., 1977; Putney and Bianchi, 1974). The degree of suppression by $2 \times 10^{-5}$ M dantrolene on the myocardium, however, was rather mild and amounted to about 31% of the control twitch amplitude. This contrasted to the drug effect as reported on the skeletal muscle showing the stronger inotropic action at a lower concentration than the present one (Ellis et al., 1976; Meyler et al., 1976). It was also shown that the drug depressed the twitch tension without any influences on delayed afterdepolarizations, aftercontractions, and transient inward current developing in the low-$K^+$, high-$Ca^{2+}$ solution. The latter three activities are assumed to reflect cyclic changes in internal $Ca^{2+}$ caused by the $Ca^{2+}$-induced $Ca^{2+}$-release from the stores (Kass et al., 1978).

There have been variable effects of dantrolene on the mechanical and electrical properties of cardiac preparations (Hatae et al., 1980; Honerjäger and Alischewski, 1983; Mészáros et al., 1981, 1982; Salata and Jalife, 1982; Salata et al., 1983). These different results might result, from differences in tissue, drug concentrations used, solvents, and methodology employed with individual experiments. Dantrolene has recently been shown to have a stimulative action on sympathetic nerve endings distributed in the myocardium (Honerjäger and Alischewski, 1983; Mészáros et al., 1982). This action became prominent at high concentrations of 100–200 $\mu$M. Thus, the positive inotropic effects of the drug can be explained by this indirect action. To ascertain the inotropic effect of the drug, slow response activity was used as a tool in most of the reports (Hatae et al., 1980; Honerjäger and Alischewski, 1983; Mészáros et al., 1981, 1982; Salata and Jalife, 1982; Salata et al., 1983). A stable slow response, however, is rather difficult to obtain (see Ehara and Inazawa, 1980), since it responds with variable amplitudes to electrical stimulation and with frequency variations because it requires a high strength of stimulation. The latter factor might promote the stimulation of sympathetic nerve terminalis distributed in the preparations (Blinks, 1966). Therefore, the results obtained with the slow response activity are prone to be influenced by factors other than the drug action. Dantrolene is a relatively insoluble agent to physiological solutions, and it is necessary to dissolve the drug in a certain kind of solvents for using in vitro study. We used a low concentration of methanol as a solvent, and its concentration was less than the threshold of its negative ino-
tropic action. Therefore, it seems reasonable to conclude that a decrease in the contractile strength while applying dantrolene in the present experiments was a direct action of the drug. It is probably due to the limited time of the treatment (20 min) that the inotropic effect of dantrolene in the Tyrode's solution was reversible on the wash-out with drug-free solution. When longer exposure times or higher concentrations than the present ones were used, no reversibleness of the drug effect was reported (Honérjäger and Alischewski, 1983; Salata and Jalife, 1982; Salata et al., 1983).

Honérjäger and Alischewski (1983) have recently reported that slow action potentials in guinea-pig papillary muscles from reserpine-pretreated animals or in muscles exposed to tetrodotoxin were unaffected by dantrolene with respect to the $V_{\text{max}}$ and overshoot. They concluded that dantrolene had no appreciable effect on the slow inward current. Slow action potentials, however, are indirect measures of the slow inward current and are also affected by changes of the outward current (Reuter, 1979). In the present experiments, dantrolene did not affect the plateau duration of ventricular action potentials and showed no influence on the slow inward current directly measured by the voltage clamp technique. Therefore, our results support the observations reported by Honérjäger and Alischewski (1983) and provide direct evidence of their conclusion. In the present study, 90% APD, showed no prolongation and the outward current on depolarization was barely affected during the application of $2 \times 10^{-5}$ M dantrolene for 20 min, while other investigators observed prolonged APD when a higher concentration than the present one was used, or the drug was applied to Purkinje fibers (Honérjäger and Alischewski, 1983; Salata and Jalife, 1982; Salata et al., 1983). It cannot be ruled out, therefore, that dantrolene depresses the outward K+ current when a higher concentration than $2 \times 10^{-5}$ M is used, or the drug is applied to Purkinje fibers. Although dantrolene showed no effect on the slow inward current and a negligible influence on the outward current in our preparations, the drug did yield a negative inotropic effect. This suggests that the major site of the drug action is not on the sarcolemma but in the intracellular location, since it has also been shown no action on the Na+-Ca2+ exchange mechanism (Honérjäger and Alischewski, 1983).

Kass et al. (1978) proposed that delayed afterdepolarizations were caused by a process of a cyclic release of Ca2+ from the intracellular store occurring when the cells were Ca2+-overloaded. A cyclic increase in intracellular Ca2+ produces ACs at the contractile machinery and conductance changes at the sarcolemma, which allowed the flow of the transient inward current forming DADs (Lederer and Tsien, 1976). This hypothesis is based in part on the observation made by Fabiato and Fabiato (1975) that Ca2+-overload could induce a Ca2+-induced Ca2+-release in skinned cardiac cells. The hypothesis has also been supported by the findings that the Ca2+-injection into isolated ventricular myocytes caused an appearance of the TI and injection of EGTA eliminated it (Matsuda et al., 1982).
The application of digitalis and high extracellular calcium concentration provide the condition of Ca\(^{2+}\)-overload (Kass et al., 1978). Low external potassium has also been shown to cause the development of DADs (Eisner and Lederer, 1979) due to the block of the Na\(^+\)-K\(^+\) pump and resultant accumulation of internal sodium which raises internal calcium concentration through the Na\(^+\)-Ca\(^{2+}\) exchange mechanism (Reuter and Seitz, 1968). Therefore, it seems appropriate to assume that the DADs as observed in our preparations were brought about by the same mechanism as that in Purkinje fibers exposed to digitalis.

In our experiments, dantrolene decreased the twitch amplitude without any changes in the DAD's and in the AC's amplitudes. For the dissociation of the effects on the twitch tension and the ACs, it seems unlikely that dantrolene acts on the contractile machinery directly, but rather suggests that the drug affects the process of the excitation-contraction coupling, probably at the Ca\(^{2+}\)-releasing process in heart muscle and similarly in its action in skeletal muscle (Brocklehurst, 1975; Desmedt and Hainaut, 1977; Ellis and Carpenter, 1972; Pinder et al., 1977; Putney and Bianchi, 1974; Takauji et al., 1975; Yamamoto et al., 1977; Van Winkle, 1976). It is generally believed that two types of Ca\(^{2+}\)-releasing processes, Ca\(^{2+}\)-induced Ca\(^{2+}\)-release and depolarization-induced Ca\(^{2+}\)-release, operate in the contraction of skeletal as well as cardiac muscles (Endo, 1977; Fabiato, 1983). Although the Ca\(^{2+}\)-induced Ca\(^{2+}\)-release is assumed to be an important factor in the excitation-contraction coupling, its precise role in the contraction of intact heart muscle under a physiological condition has not yet been clarified. If the idea proposed by Kass et al. (1978) was correct, the present results may indicate that dantrolene does not act on the Ca\(^{2+}\)-induced Ca\(^{2+}\)-release occurring in the Ca\(^{2+}\)-overloaded cells. Since dantrolene depressed the twitch in the normal cells, a single mechanism of the action operable in both conditions can be considered. The possible action of dantrolene is, thus, suggested to affect the process of depolarization-induced Ca\(^{2+}\)-release from the sarcoplasmic reticulum directly, or indirectly by impairing the triggering mechanism which links sarcolemmal depolarization to the release of Ca\(^{2+}\) from the sarcoplasmic reticulum. There might be another possibility of the action of dantrolene, since it prolonged the relaxation time of the twitch (Table 1) and eliminated positive-staircase phenomenon during the train impulses (Fig. 4). These results may indicate that dantrolene inhibits the Ca\(^{2+}\)-uptake to the sarcoplasmic reticulum. In skeletal muscle, however, the dantrolene-induced decrease in twitch tension has been shown to occur in association with a reduction of the intracellular Ca\(^{2+}\) transient (Lopez et al., 1979).

Our results reveal that dantrolene has a different action on the excitation-contraction coupling from that of ryanodine, which has a striking inotropic effect in skeletal and cardiac muscle (Jenden and Fairhurst, 1969) and has been shown to affect the calcium metabolism by the sarcoplasmic reticulum (Sutko and Willerson, 1980). Ryanodine has recently been shown to prevent the oscillations in intracellular calcium that activate the TI and the AC by inhibiting the release of
calcium from the sarcoplasmic reticulum under K⁺-free conditions (Sutko and Kenyon, 1983).

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