Amantadine-Induced Diastolic Depolarization and Automaticity in Ventricular Muscle

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SUMMARY. We studied the cardiac effects of amantadine, an antiviral and anti-Parkinson drug. Amantadine hydrochloride (100-800 μM) produced significant changes in the electrophysiological properties of isolated ventricular muscle preparations from frog, rabbit, cat, dog, and calf. At relatively low concentrations (100-300 μM), the drug increased action potential duration, decreased action potential amplitude and maximum diastolic potential, and induced phase 4 depolarization. Amantadine also caused subthreshold diastolic depolarizations, apparent upon cessation of stimulation in all preparations studied. The amplitude of the diastolic depolarizations increased as a function of time and/or concentration of drug, eventually reached threshold, and spontaneous activity ensued. In the steady state, amantadine-induced spontaneous activity was rather stable, and the rate was dependent upon the amantadine and external potassium concentrations, as well as the membrane potential. In the absence of stimulation, amantadine-induced spontaneous activity occurred abruptly or could be triggered by a single stimulus, often occurring in a "bursting" fashion that appeared to originate from multiple discrete foci. All actions of amantadine were rapidly reversed upon washout. Propranolol had no effect on the actions of the drug. Amantadine-induced spontaneous activity was unaffected by lidocaine, diminished by TTX, and reduced or abolished by verapamil. The results indicate that amantadine can directly alter the membrane properties of ventricular muscle, possibly due to an effect on potassium conductance. Furthermore, amantadine can be used as a tool to study the ionic basis of ventricular automaticity and to model cellular mechanisms of ventricular rhythm disturbances. (Circ Res 51: 722-732, 1982)

AMANTADINE HYDROCHLORIDE (1-adamantanamine hydrochloride, Symmetrel) is an antiviral agent that is somewhat effective in the treatment of Parkinson's disease (Schwab et al., 1969). The known ability of amantadine to increase the synthesis and release of dopamine from dopaminergic neurons in the corpus striatum (Scatton et al., 1970; Voigtlander and Moore, 1971) is one probable mechanism for its symptomatic relief in Parkinsonism. In addition, amantadine has been shown to block neuromuscular transmission by reducing the response of the post-junctional membrane to acetylcholine (Nastuk et al., 1976). This inhibition is voltage-dependent and appears to result from the reaction of amantadine with an ionic channel-modulator protein rather than with the acetylcholine receptor itself (Albuquerque et al., 1978). Furthermore, the electrophysiological properties of the sarcolemmal membrane are significantly altered during exposure to amantadine for relatively long periods of time. Amantadine induces partial membrane depolarization followed by prolongation of the repolarization phase of the action potential, along with a decrease in the action potential amplitude and upstroke velocity (Tsai et al., 1978).

In addition to its effects in skeletal muscle, amantadine has been shown to affect the synthesis, release and uptake of catecholamines in both the central and peripheral nervous systems (Vernier et al., 1969; Greulak et al., 1970; Farnebo et al., 1971), and recently it was shown to increase the amplitude of ouabain-induced oscillatory afterpotentials (OAP) in guinea pig ventricular papillary muscles (Karagueuzian and Katzung, 1981). The OAP-potentiating effect of amantadine was attributed to its ability to release catecholamines. Since amantadine has been shown to affect directly the properties of nerve and skeletal muscle, it is possible that the strong OAP-potentiation may be related, at least in part, to a direct effect of amantadine on the electrical properties of ventricular muscle. The present study was undertaken to investigate this possibility.

Our experiments demonstrate that amantadine produces major alterations in transmembrane potential characteristics of isolated ventricular myocardial tissues. These findings may have clinical implications and suggest that amantadine may be used as a pharmacological tool for the study of time- and voltage-dependent ionic mechanisms in cardiac electrophysiology, as well as to generate experimental models of cardiac rhythm disturbances.

Methods

Animals and Preparations

We studied the effects of amantadine in vitro on ventricular muscle preparations from a variety of species: frog, rabbit, cat, dog, and calf. Frogs, Rana pipiens, were pithed and their hearts were removed and dissected in cool oxy-
genated Ringer's solution. Thin strips of muscle (approximately 1 mm wide, 1 mm deep, and 5–10 mm long) were dissected from the epicardial surface of the ventricle. Frog preparations were superfused at a rate of 8 ml/min with oxygenated Ringer's solution, pH 7.0–7.2, at room temperature (22–24°C). The composition of the Ringer’s solution was (mM): Na+, 110; K+, 2.0; Cl−, 96; HCO3−, 20; Ca++ , 1.8; Mg++, 2.0; dextrose, 5.6;

Calf hearts were obtained from the local slaughterhouse and placed in cool, oxygenated Tyrode's solution for later dissection. Adult mongrel dogs (10–20 kg), mongrel cats (1.5–3.0 kg), and New Zealand white rabbits (1.5–3.0 kg) of either sex were anesthetized with sodium pentobarbital (35 mg/kg, iv), and their hearts were rapidly removed. Papillary muscles were oriented with the tendinous tip (1.0–1.5 mm) in the test chamber. All three compartments were perfused with standard Tyrode’s solution during a 1-hour equilibration period. Afterward, the middle chamber (2) was perfused with isotonic sucrose solution (300 mM) containing 5 mM glucose, 0.1 mM CaCl2, and gassed with 100% O2. Chamber 3 was perfused with 20 mM K+ Tyrode’s. The test compartment (1) was continually perfused with standard Tyrode’s solution, or solutions containing drugs. To prevent short-circuiting, transmembrane potentials were recorded differentially from the segment in chamber 1. Current pulses were passed through the fiber using Ag-AgCl electrodes placed in chambers 1 and 3 and coupled to a Frederick Haer P6I unit, programmed to deliver current pulses (2–40 μA) between 3 and 10 seconds long.

Chemicals

Amantadine hydrochloride (Sigma) was dissolved at a concentration of 0.1 M (18.8 mg/ml) in deionized water, and appropriate portions of this stock solution were added to the Tyrode’s solution just prior to use to achieve final amantadine concentrations. Tetrodotoxin (TTX; Sigma) was dissolved directly in the Tyrode’s solution at a concentration of 30 μM (1 mg/100 ml).

Statistical Analysis

Statistical analyses of drug-induced changes in action potential characteristics (Table 1) were performed using analysis of variance. Comparisons of individual drug treatment means to a common control were performed using Dunnett’s t-test (Steel and Torrie, 1960).

Results

Effects on Action Potential Morphology

Within minutes after addition of amantadine (100–800 μm) to the superfusion medium, we observed major changes in the electrophysiological properties

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>MDP (mV)</th>
<th>TOP (mV)</th>
<th>APA (mV)</th>
<th>APD90 (msec)</th>
<th>APD10 (msec)</th>
<th>Vmax (V/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−84.1 ± 2.0</td>
<td>−82.5 ± 2.3</td>
<td>109 ± 9</td>
<td>133 ± 9</td>
<td>172 ± 10</td>
<td>136 ± 12</td>
</tr>
<tr>
<td>Amantadine</td>
<td>−83.3 ± 1.3</td>
<td>−81.0 ± 1.3</td>
<td>110 ± 1</td>
<td>150 ± 12</td>
<td>196 ± 14</td>
<td>122 ± 14</td>
</tr>
<tr>
<td>100 μM</td>
<td>−77.3 ± 1.8</td>
<td>−72.4 ± 4.2</td>
<td>100 ± 4</td>
<td>161 ± 15</td>
<td>221 ± 24</td>
<td>109 ± 13</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM; n = 7; BCL = 600 msec.

* P < 0.05; † P < 0.01, analysis by Dunnett’s method.
of myocardial tissues from all species studied. Figure 1 shows superimposed transmembrane potential recordings to illustrate the effects of amantadine (200 μM) on action potential morphology in a calf ventricular trabecula stimulated continuously at a BCL of 2000 msec. Control action potentials had the following characteristics: MDP = −93 mV, APA = 123 mV, APD90 = 181 msec, APD90 = 215 msec. No phase 4 depolarization was observed during a control period of 1 hour. As illustrated by the superimposed traces, exposure to amantadine induced progressive changes in all of these measurements. After 20 minutes of superfusion, APA decreased to 112 mV while APD50 and APD90 increased to 210 and 252 msec, respectively. These changes became more pronounced at 40 and 60 minutes, at which time the tissue had depolarized to a MDP of −90 mV.

In addition to these changes (Fig. 1), amantadine induced a gradual decrease in the TOP of this cell, associated with slow diastolic depolarization that became progressively steeper during 1 hour of continuous drug superfusion. In this preparation, while the MDP was decreased only by about 3 mV, the level of the TOP changed from −93 mV in the control, to −90, −87, and −85 mV at 20, 40, and 60 minutes of amantadine superfusion, respectively.

Analogous results were obtained with tissue from other species. Table 1 summarizes the effects of amantadine (100 and 200 μM) in a series of seven cat papillary muscle preparations driven continuously at a BCL of 600 msec. Superfusion with amantadine (15 minutes) produced concentration-dependent decreases in MDP, TOP, APA, and Vmax and increases in APD50 and APD90. The change in TOP (200 μM amantadine) was statistically significant (P < 0.05) and was greater than the change in MDP, indicating the development of diastolic depolarization. Also significant (P < 0.05) were the decrease in APA after 15 minutes of exposure to 200 μM amantadine.

**Amanadine-Induced Spontaneous Activity**

Since amantadine has been shown to potentiate digitalis-induced oscillatory afterpotentials (OAP's) (Karagueuzian and Katzung, 1981), it is conceivable that amantadine itself can induce oscillatory activity during diastole, leading to automaticity in ventricular muscle. To investigate this hypothesis and to rule out the possibility of Purkinje fibers being responsible for the drug-induced effects, we used isolated ventricular preparations that do not contain specialized conduction tissues and, under normal circumstances do not undergo phase 4 depolarization (see Methods).

Amanadine consistently induced slow diastolic depolarization in continuously beating ventricular muscle. The time course and the characteristics of these diastolic depolarizations were studied using trains of 10 or 20 stimuli and BCL's that ranged between 500 and 2000 msec, followed by long (5 to 40 seconds) rest intervals. Figure 2 shows the time course of effects found in a frog epicardial preparation. Each trace starts with the last four of a series of 10 action potentials (BCL 2000 msec) and ends after a period of 22 seconds, during which no stimulation was applied. During 1 hour of control (panel A), there was no phase 4 depolarization, and MDP remained stable at about −92 mV. Panel B shows recordings from the...
same cell after 15 minutes of exposure to 800 μm amantadine, at which time the cell depolarized to a MDP of −85 mV. Upon termination of the train, the cell membrane underwent a slow diastolic depolarization that reached a peak amplitude of 8 mV after an interval of 3.3 seconds and decayed progressively over a much longer time course. This subthreshold diastolic depolarization (SDD) appeared within 3–5 minutes after addition of amantadine and increased in amplitude and time-to-peak at later stages or higher drug concentrations. After 20 minutes (panel C), the SDD reached its peak amplitude of 14 mV at about 5.7 seconds. At 30 minutes (panel D), the diastolic depolarization reached threshold, producing two spontaneous beats (BCL = 7760 and 8190 msec) at a MDP of −77 mV. The spontaneous discharges increased in frequency and number until, at 35 minutes (panel E), automatic activity became rhythmic and continuous (see also Fig. 3). All effects of amantadine were rapidly reversible upon washout.

This general pattern of effects occurred consistently and reproducibly in all experiments, including ventricular muscle preparations from five frogs, five rabbits, eight cats, five dogs and three calves.

Does the Amantadine-Induced Diastolic Depolarization Result from Catecholamine Release?

Amantadine has been shown to increase the release of catecholamines from sympathetic nerve terminals (see Discussion), and its potentiating effect on digitalis-induced OAP’s in papillary muscles has been attributed to this action (Karagueuzian and Katzung, 1981). Since, under certain conditions, large concentrations of β-adrenergic agonists can induce spontaneous activity in ventricular tissues (Greiner and Garb, 1950; Katzung et al., 1975), it is possible that the amantadine-induced effects were mediated indirectly by a release of catecholamines from nerve terminals.

We investigated this possibility in 10 experiments in which the effects of amantadine were studied in the presence of propranolol. One such experiment in a calf ventricular trabecula is shown in Figure 3 (see also Fig. 8). Under control conditions (panel A), the MDP was −93 mV. Exposure to amantadine (200 μM) produced electrophysiological changes almost identical to those previously described. Notably, after 30 minutes (panel B), the MDP had decreased to −89 mV and SDD’s had developed. As usual, the SDD amplitude became progressively larger, with peaks occurring at longer intervals; 10 minutes later (panel C), SDD amplitude was greatly increased. Amantadine was increased to 250 μM, and after 10 minutes (panel D), all depolarizations reached threshold and spontaneous activity ensued at a BCL of 4700 msec. Clearly, prior treatment with 2 μM propranolol had no effect on the development of amantadine’s effects on ventricular muscle. Furthermore, in three preparations, addition of propranolol after development of amantadine’s effects had no effect on the SDD’s or on the spontaneous rate.

Voltage-Dependence of Spontaneous Activity

The rate of spontaneous activity produced by amantadine was dependent upon the membrane potential. Figure 4 shows transmembrane potential (top traces) and current injection (bottom traces) recordings from a rabbit papillary muscle mounted in a sucrose gap chamber. Under control conditions (panel A1), this muscle had a resting membrane potential of −95 mV and was stable for 2 hours, displaying no spontaneous activity. Application of 3.75-second depolarizing current pulses of 28 and 31 μA in panels A2 and A3, respectively, depolarized the muscle to MDP’s of −34 and −22 mV. Passage of current produced cathodal make and anodal break excitations. However, in this preparation we were unable to in-
FIGURE 4. Voltage-dependence of amantadine-induced spontaneous activity. Top traces are transmembrane potentials, and bottom traces are current recordings obtained from a rabbit papillary muscle mounted in a sucrose gap chamber. Panel A is control. Panel B shows traces after 30 minutes of exposure to 200 μM amantadine. Panel C is 30 minutes after washout of the drug. See text for further details.

Effects of Extracellular Potassium Concentration \([K^+]_o\)

The rate of spontaneous activity induced by amantadine in ventricular muscle preparations was also dependent on \([K^+]_o\). Composite results from an experiment in a rabbit papillary muscle are shown in Figure 5. At 4.0 mM \([K^+]_o\), this preparation exhibited stable spontaneous activity for 90 minutes in the presence of 400 μM amantadine. The spontaneous BCL increased linearly as \([K^+]_o\) was varied from 2.0 to 6.0 mM. Similar results were obtained in three other experiments.

**Effects of Lidocaine, Verapamil, and TTX**

Lidocaine caused no consistent change in amantadine-induced spontaneous activity. In Figure 6A, after 30 minutes of amantadine (250 μM), this calf trabecula stabilized at a MDP of -79 mV and was beating spontaneously at a BCL of 3770 msec. In Figure 6B after 30 minutes of superfusion with 10 μM lidocaine in the continued presence of 250 μM amantadine, there was a slight increase in the rate of amantadine-induced automaticity (BCL = 3640). Under similar conditions in three other experiments, 10 μM lidocaine produced either no change or a slight decrease in the rate of amantadine-induced spontaneous activity.

In contrast, addition of verapamil to amantadine-treated ventricular muscle preparations rapidly abolished spontaneous activity. The records in Figure 6C were taken after 30 minutes of lidocaine washout. The effect of 2 μM verapamil is shown 5 minutes after addition to the superfusing solution. The MDP further decreased to -69 mV and the BCL was greatly increased to 7140 msec. In panel D, after 10 minutes of verapamil, MDP was -61 mV and the BCL was 10,700 msec. Thirty seconds later, spontaneous activity ceased altogether. Verapamil rapidly and consistently abolished amantadine-induced spontaneous activity in five other preparations of mammalian ventricle (rabbit and cat) and one frog epicardial preparation. On two occasions, verapamil produced a further de-
Effects of Amantadine on the Heart

Figure 6. Effect of lidocaine and verapamil on amantadine-induced spontaneous activity. Panel A: automaticity in a calf trabecula after 30 minutes of 250 μM amantadine. Panel B: after 30 minutes of 10 μM lidocaine. Following 30 minutes washout of lidocaine, the spontaneous rate greatly decreased after exposure to 2 μM verapamil for 5 minutes (panel C). The rate was slowed further after 10 minutes (panel D); spontaneous activity ceased 30 seconds later.

Figure 7. Effect of TTX on amantadine-induced spontaneous activity. All panels show transmembrane potential traces recorded at two different sites (approximately 8 mm apart) from a cat epicardial strip. Same impalements maintained throughout. Panel A: spontaneous activity after 2 hours of exposure to 250 μM amantadine. Addition of TTX produced rapid changes in spontaneous activity after 2, 3, and 3.2 minutes of exposure in panels B, C, and D, respectively. Panel E: 30 minutes after exclusion of TTX from Tyrode’s.

During amantadine (250 μM) superfusion, a discrete pacemaker focus located close to the top recording site had developed, and generated impulses at a relatively stable frequency for 1 hour (panel A). The BCL was 885 msec and MDP’s were -86 and -89 mV in the top and bottom traces, respectively. In panel B, 2 minutes after the addition of TTX (30 μM), the spontaneous BCL increased to 1320 msec. This was associated not with a decrease in the slope of phase 4 depolarization but with a change in threshold and TOP which became less negative in both cells. In fact, the slope of phase 4 depolarization in the distal cell (bottom panel) became steeper, and pacemaker dominance shifted to this site. At 3 minutes (panel C), spontaneous activity stopped abruptly, terminating with a subthreshold oscillation which originated nearer the bottom recording site. However, the preparation did not become totally quiescent; rather, after several seconds (panel D), rhythmic subthreshold oscillatory activity appeared. These oscillations probably represent spontaneous discharges from a localized focus which, in this example, activated the rest of the preparation in a 3-to-1 manner. This type of activity persisted for the remainder of the 15 minutes of TTX superfusion, but was reversed very rapidly during washout. Panel E shows records obtained 30 minutes after TTX was excluded from the Tyrode’s. The preparation rapidly resumed its amantadine-induced ac-
tivity which, after 1 hour (not shown) became focalized and conditions returned to the original control (panel A).

These experiments show that TTX is capable of diminishing or abolishing amantadine-induced pacemaker-activity indirectly by decreasing the availability of the sodium-carrying system responsible for the upstroke of the action potential, thereby diminishing the likelihood of propagation from the amantadine-induced dominant pacemaker.

**Some Properties of Amantadine-Induced SDD's**

In preparations driven by external stimulation, amantadine-induced spontaneous activity was always preceded by the development of subthreshold diastolic depolarizations, which are further characterized in Figure 8. Panel A shows polygraph recordings of transmembrane potentials obtained from a cat papillary muscle stimulated with trains of 21 beats (BCL = 500 msec) separated by 20-seconds pauses. Control MDP was —85 mV (panel A). In panel B, 5 minutes of exposure to amantadine (200 μM) was sufficient to induce a SDD which reached a peak of 4 mV, 1.0 second after the last stimulus. The amplitude and time to peak of the SDD increased progressively until, at 15 minutes (panel C), the amplitude was 17 mV. In panel D, after only 15 minutes of washout, there was almost a complete return to control conditions.

A common feature of amantadine-induced effects was the appearance of low amplitude oscillations superimposed upon the SDD’s. In conjunction, the peak amplitude of the SDD’s in some preparations was found to be inversely related to the frequency of stimulation. In Figure 8, after 15 minutes of 200 μM amantadine, changing the stimulus BCL to 500, 600, and 700 msec in panels E, F, and G, respectively, produced SDD peak amplitudes of 17, 20, and 23 mV. Upon increasing the BCL to 800 msec (panel H), the diastolic depolarization reached threshold and was followed by spontaneous activity for 21 successive beats. Frequency dependence was studied in six experiments; in three of these we observed similar relationships to those depicted in Figure 8. There was no frequency dependence in the other experiments.

In three of six experiments, the amplitude of the SDD was also inversely related to the number of stimuli in the train. Panels I–L of Figure 8 illustrate this inverse relationship. The preparation was stimulated with trains of variable duration at constant BCL of 800 msec. In panel I, a train of five stimuli was followed by two spontaneous discharges and a SDD. In J, K, and L, trains of 10, 15, and 20 beats, respectively, did not trigger spontaneous discharges but were followed by single SDD’s of diminishing amplitude.

There were no statistically significant correlations between train duration or frequency and SDD amplitude. As such, these results may be related to cycle-length-dependent variations in conduction of responses triggered by the SDD in an initially small focus of automaticity, rather than an intrinsic frequency dependence of SDD amplitude. Such an ex-

![Figure 8](http://circres.ahajournals.org/)

**Figure 8.** Effect of frequency and number of driven action potentials on amantadine-induced SDD’s. Panel A shows control transmembrane potential recordings from a cat papillary muscle stimulated at a BCL of 500 msec. Propranolol (2 μM) present throughout. Panel B, 5 minutes and panel C, 15 minutes after addition of 200 μM amantadine. Panel D: 15 minutes after washout of amantadine. Panels E–L are 15 minutes after readdition of 200 μM amantadine. Trains of 20 driven beats at BCL’s of 500, 600, 700, and 800 msec are shown in panels E, F, G, and H, respectively. Stimulated trains of 5, 10, 15, and 20 beats in panels I, J, K, and L, respectively, at a constant BCL of 800 msec.
Spontaneous "Bursting"

A common feature of the amantadine effects in ventricular muscle was that, initially, in otherwise quiescent preparations, spontaneous activity occurred in bursts of high frequency discharges separated by long periods of silence. A striking example is illustrated in Figure 10, obtained from a dog right ventricular trabecula. Following a stable control period of 1 hour (not shown), amantadine (200 μM) was added to the Tyrode's. Panel A shows polygraph traces of transmembrane potentials recorded at 15 minutes. The stimulator was turned off (arrow) and the membrane hyperpolarized beyond the stimulated control (−2 mV), but then very gradually depolarized. After 2 minutes of continuous slow diastolic depolarization, the cell suddenly reached threshold and fired a series of 11 spontaneous discharges. The rate of discharge was rapid at first but gradually decreased until, after the 11th discharge, the cell failed to reach threshold. Activity terminated with a relatively small SDD. In the following minutes, bursting occurred at somewhat regular intervals, appeared to be multifocal (panels B and C), and was of variable frequency, depending on the point of origin. At 25 minutes (panel B), bursting predominated at a relatively slow frequency, with spontaneous activity originating at the site that appeared to be near the impaled cell. This is suggested by the smooth transition from phase 4 depolarization to action potential upstroke in the individual discharges. After the third burst in panel B, activity suddenly shifted to a different focus at a distance from the impaled cell. This focus discharged "prematurely" at a much faster frequency, terminated abruptly (no apparent slowing) after the eighth discharge, and was followed by a small SDD. The timing of the following burst appeared to be delayed, but, thereafter, rhythmic "slow" frequency bursting resumed. At 30 minutes (panel C), "fast" frequency bursting became predominant, but soon after, dominance shifted back to the slower focus. This pattern continued for several minutes with a gradual increase in the number of discharges per burst, and a concomitant decrease in the interval between bursts until, at 40 minutes (panel D), continuous spontaneous activity ensued.

It is impossible to tell from this experiment whether the fast frequency bursting was generated by spontaneous or "triggered" activity in subendocardial Purkinje fibers which may have been present in this preparation. However, multifocal bursting similar to that in Figure 10 occurred in six other experiments (two canine, four feline), three of which were performed using thin strips of left ventricular epicardium. These include the experiment in Figure 7, the records of which demonstrate the multifocal nature of the activity.

Discussion

Amantadine has direct effects on the electrophysiological properties of ventricular muscle. At relatively low concentrations, it induces a decrease in the MDP and a larger decrease in the TOP associated with the development of phase 4 depolarization. The drug also increases APD and decreases APA (Fig. 1; Table 1). This latter change may well be secondary to membrane depolarization. This idea is supported by the fact that the decrease in APA can display a linear
Amantadine SDD's vs. Digitalis OAP's

Amantadine-induced SDD's differ from digitalis-induced OAP's in a number of ways (see Ferrier, 1977). In most cases, SDD's are characterized by a relatively rapid depolarization followed by a slow approach to the resting potential (Figs. 2 and 3); digitalis-induced afterpotentials are usually oscillatory and, in many published examples, more than one OAP occurs in sequence at a relatively high frequency. The amplitude and coupling interval of SDD's increase as a function of time and drug concentration; OAP amplitude increases with time and cardiac glycoside concentration, but the coupling interval is relatively constant. Amantadine-induced SDD's are not associated with any contractile event (Fig. 9); digitalis-induced OAP's are usually associated with oscillatory after-contractions. Amantadine-induced SDD's and spontaneous discharges can occur in a frequency-dependent manner opposite to that of digitalis-induced OAP's (Fig. 8). In this respect, amantadine-induced diastolic depolarization resembles "normal" phase 4 depolarization in Purkinje fibers, because it can be suppressed by rapid overdrive. All these properties suggest that amantadine-induced diastolic depolarizations are related to a mechanism other than that responsible for oscillatory afterpotentials during digitalis intoxication.

Amantadine (200 μM) markedly increased the amplitude of ouabain-induced OAP's in guinea pig papillary muscles (Karagueuzian and Katzung, 1981), an effect attributed to the ability of the drug to release catecholamines. We have not studied the effects of amantadine on digitalis oscillations. However, in light of the present observations, a reconsideration of the mechanism by which amantadine potentiates ouabain-induced OAP's is in order. If this OAP potentiation is mediated by a release of catecholamines, then correlation with the decrease in TOP (Salata and Jalife, unpublished data). Another striking effect produced by amantadine was the development of SDD's (Figs. 2, 3, 8, and 9). All effects increased in magnitude as a function of time and drug concentration. In addition, as the diastolic depolarizations became larger, they eventually reached threshold and produced spontaneous pacemaker discharges. The rate of spontaneous activity increased progressively, as the membrane potential gradually declined, reaching a steady-state after 40 to 60 minutes.

It is noteworthy that amantadine-induced spontaneous activity often appears after only relatively small decreases in the resting membrane potential (Fig. 10). Automaticity in ventricular muscle at such high levels of membrane potential, to our knowledge, has not been reported previously.

The overall pattern of results was seen consistently; however, amantadine-induced effects displayed a species dependence. As a general rule, frog ventricular preparations required higher concentrations and/or longer periods of exposure for the effects to become manifest. Among the mammals studied, rabbit cardiac muscle was least sensitive, while cat, dog and calf myocardium had essentially the same sensitivity. In all species, effects were completely reversible by superfusion with drug-free solution within 45 minutes. Amantadine's actions were not mediated by the release of catecholamines from nerve terminals or by an interaction with β-adrenergic receptors. Propranolol did not prevent or reverse the effects.

Amantadine produced automatic activity in a variety of ventricular muscle preparations, including epicardial strips from frogs, cats, and dogs, indicating that the action was not mediated by Purkinje fibers. We surmise that these effects are related to a direct action of the drug on the membrane of the ventricular muscle cell.

FIGURE 10. Spontaneous "bursting." Transmembrane potential recordings from a canine trabecula. Stimulator turned off at arrow. Exposure to amantadine (200 μM) for 15, 25, 30, and 40 minutes in panels A, B, C, and D, respectively.
the accompanying OAC's should also be increased. There is no evidence for such an effect. Furthermore, amantadine actually produces a decrease in the amplitude (Fig. 9) and rate of relaxation of isometric contraction, the reverse of the well-known effects of catecholamines (Katz, 1977). Accordingly, the ability of amantadine to produce SDD's directly may be an important factor in its potentiation of ouabain-induced OAP's.

Mechanisms of Spontaneous Activity

At the present time, we can only speculate about the mechanism of amantadine-induced automaticity in ventricular muscle. Thus far, no voltage clamp data are available on the effects of amantadine on transmembrane currents in cardiac tissues. Accordingly, analysis of these changes in terms of ionic mechanisms is based on indirect evidence.

In skeletal muscle, amantadine has strong postsynaptic action related directly to a partial membrane depolarization, followed by a prolongation of the falling phase of the action potential and development of spontaneous muscle contracture. The membrane depolarization is induced by amantadine at concentrations that cause marked effects on end-plate currents and the action potential; it is about 20 mV in rat or frog muscles. Such effects may be due to a partial block of the K+ conductance (Tsai et al., 1978).

Our results show that amantadine can induce spontaneous activity over a wide range of membrane potentials, including MDP's as high as —85 mV (Fig. 10) or as low as —30 mV (Fig. 4). Furthermore, the rate of spontaneous activity produced by this agent is voltage dependent, and is abolished by verapamil, but it can also be affected by TTX, in a manner which is dependent on the membrane potential (Fig. 7). This indicates that, whatever the mechanism of slow diastolic depolarization, spontaneous discharges can be sustained either by the fast sodium current (MDP > —70 mV) or by the slow inward current in those preparations in which the fast sodium channels have been completely inactivated by depolarization.

The proposed mechanisms for automatic activity in depolarized ventricular myocardium have been reviewed recently by Surawicz (1980). Pacemaker activity can be induced in ventricular muscle by means of a variety of manipulations. These include treatment with barium salts (Antoni and Oederisse, 1965; Reid and Hecht, 1967), aconitine (Heistracher and Pillat, 1962), papaverine (Greiner and Garb, 1950), and the application of excessive stretch (Kaufman and Theophile, 1967). Automatic activity has been shown to appear also in calf and sheep ventricular trabeculae superfused with K+- and Ca++-free solution (Muller, 1965). Monkey papillary muscles mounted in a sucrose gap spontaneously depolarized and became automatic following strong hyperpolarizing current pulses of long duration (Antoni and Taegtmeyer, 1965). In similar fashion, spontaneous activity can be produced by application of depolarizing current under a variety of conditions in monkey (Antoni, 1970) and guinea pig (Katzung, 1974, 1975; Imanishi and Surawicz, 1976) papillary muscles. The rate of depolarization-induced automaticity is dependent upon membrane potential and [K+]o, and amantadine-induced effects resemble it in this and other ways (Katzung et al., 1975; 1977).

In many respects, the actions of amantadine on ventricular muscle also show marked similarities to the effects of barium (Ba++) in cardiac tissues. Perfusion with Ba++ also induces prolongation of the action potential pacemaker activity, and progressive depolarization in atrial and ventricular muscle (Reid and Hecht, 1967; Toda, 1970). These changes have been attributed in part to a nonspecific decrease in potassium conductance (Sperelakis, 1972), but recent experiments in Purkinje fibers suggest that at relatively low concentrations (5 mM) Ba++ can reduce the time-independent outward current component due to I K with no significant effect on the pacemaker current (I p; DiFrancesco, 1981). Thus, it is conceivable that, in ventricular muscle, the effects of Ba++ and those of amantadine are mediated by a selective reduction in the time-independent current I K and by an increased dependence in the transmembrane potential on I K, which, in the presence of sufficient background inward current, can lead to an increase in the action potential duration and to slow diastolic depolarization. Alternatively, it is tempting to speculate that, by partially blocking the dominant diastolic potassium conductance, amantadine may unmask an as yet hypothetical "pacemaker current" in ventricular muscle. This current would be similar to I K, would operate in the potential range of —90 to —60 mV, and would lead to diastolic depolarization from MDP's as large as —90 mV. However, proof or disproof of this hypothesis awaits further amantadine experiments using perturbation and voltage-clamp techniques.

Clinical Implications

The clinical implications of this study are uncertain. To our knowledge, there is no evidence for induction of arrhythmias at therapeutic levels of amantadine. Since amantadine is frequently used in elderly patients to protect them from influenza or for treatment of Parkinson's disease, the possible association with the high incidence of arrhythmias in this age group is worthy of further study. Furthermore, coincidental administration of cardiac glycosides is not uncommon in this group of patients and may enhance the likelihood of arrhythmias.

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References


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