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Effects of mexiletine on delayed after-depolarization and triggered activity

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Summary. The effects of mexiletine on delayed afterdepolarization (DAD) and triggered activity (TA) were studied in the rabbit ventricular muscle using standard microelectrode techniques. First, three kinds of perfusate (modified Tyrode's solution, K^+-free solution, and 1 m*M* K^+ + 5.4 m*M* Ca^{2+} solution) were used to see whether DAD and TA could be induced by rapid stimulations (cycle lengths of 1000, 600, and 300 ms with trains of 10 and 20 stimuli). The inducibility of DAD and TA in modified Tyrode's solution, in K^+-free solution, and in low K^+ + high Ca^{2+} solution was 0%, 8%, and 83%, respectively. The last value was significantly higher than the former values. DAD and TA were induced only by stimulation at the shortest cycle length of 300 ms, and the inducibility was significantly higher with trains of 20 than with those of 10 stimuli. When DAD and/or TA were induced, the effects of mexiletine (5 mg/l) were tested. Mexiletine totally suppressed DAD and TA in 94% of the preparations within 20 min after its addition. Abolition of DAD and TA was associated with a failure of the 1 : 1 response to the stimuli in 53% of the preparations. The drug tended to prolong the coupling interval of DAD and TA, and significantly reduced the DAD amplitude. Possible mechanisms of action are: (1) lowered intracellular Ca^{2+} concentration either via the blocking of the fast Na^+ current or the reduction of the number of action potentials; and (2) a decrease in the transient inward current due to blockage of the Ca^{2+} current.

Key words: Mexiletine–Delayed afterdepolarization – Triggered activity – K^+-free solution – High Ca^{2+} solution

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Electrophysiologic mechanisms of various tachyarrhythmias are commonly classified into enhanced physiological automaticity, development of abnormal automaticity, and disorders of conduction causing re-entry [1, 2]. Triggered activity (TA) represents one type of abnormal automaticity and has characteristics different from other mechanisms [3–9]. In experimental animals, arrhythmias resulting from TA have been observed in a variety of myocardial tissues including the atria, mitral valve, Purkinje fibers, and ventricular muscle [3–9]. However, the role played by TA in generating clinical arrhythmias is yet to be determined.

TA occurs when a delayed afterdepolarization (DAD) reaches the threshold potential. It is now widely accepted that an elevation of intracellular calcium concentration is responsible for the induction of DAD and TA [10–13], and hence, Ca^{2+} antagonists are considered to be the most effective agents against arrhythmias caused by TA [1]. In clinical practice, however, the electrophysiologic mechanism of a given arrhythmia often cannot be readily identified [9, 14, 15], and other antiarrhythmic drugs, especially of the class 1 variety, may well be administered to patients having TA-induced arrhythmias.

Consequently, in the present experiment we studied the effects of mexiletine on DAD and TA to see whether group 1B antiarrhythmic drugs can suppress arrhythmias resulting from these mechanisms.

Material and methods

Rabbits weighing 1.5 to 2.0 kg were anesthetized with intravenous pentobarbital sodium (30–35mg/kg). The chest was opened and the heart rapidly removed. Small ventricular muscle preparations were dissected, transferred to a tissue chamber, and superfused with a modified Tyrode's (control)

Table 1. Inducibility of DAD and TA

	No. of preparations studied	Induction of DAD or TA	
		10 stimuli	20 stimuli
K^+-free perfusate	25	1 (4%)	2 (8%)
1m*M* K^+ + 5.4m*M* Ca^{2+} perfusate	24	5 (21%)	20 (83%)
Total	49	6 (12%)	22 (45%)

*, $P < 0.001$

solution. The composition (m*M*/l) was: NaCl 137, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 1.0, glucose 5.0, and NaH_2PO_4 0.33, and the pH was adjusted to 7.4 with Na_2HPO_4. The solution was not bubbled with oxygen, but the PO_2 usually measured approximately 140mmHg. The preparation was stimulated by rectangular pulses (5 ms in duration and twice the threshold intensity) through a bipolar silver electrode, and transmembrane action potentials were recorded by using conventional glass microelectrodes filled with 3 *M* KCl and having resistances of 10–30 megaohms. After the stability of the transmembrane action potentials was confirmed for at least 15 min, we started the experiments according to the following protocols:

1. First, the preparation was stimulated during control perfusion with trains of 10 and 20 stimuli at cycle lengths of 1000, 600 and 300 ms, and the development of DAD and TA was tested. When neither DAD nor TA was induced, the following test perfusion was started:
2. The perfusate was switched to a K^+-free solution, and after 5, 10, and 20 min, the preparation was stimulated with the same trains of pulses as during control perfusion. When DAD or TA was observed, mexiletine (5 mg/l) was added to the perfusate and the same stimulating procedures were repeated 5, 10, and 20 min after the addition of the drug.
3. When neither DAD nor TA was induced during the K^+-free perfusion, the perfusate was further switched to a new test solution containing 1 m*M* K^+ + 5.4 m*M* Ca^{2+}. The same stimulating protocol was once again used to test for the induction of DAD and TA. When DAD or TA developed, the effects of mexiletine were evaluated as discussed above.

Statistical analyses were carried out using Student's paired *t*-tests, and *P* values less than 0.05 were considered significant.

Results

Inducibility of DAD and TA in different perfusates

DAD and TA were never produced during control perfusion. Of a total of 25 preparations superfused with K^+-free solution, one preparation developed DAD and another developed TA. Hence, DAD or TA was produced in only two preparations (8%) (Table 1). Since the preparation in which TA developed 10 min after the start of the K^+-free perfusion failed to show either DAD or TA after 20 min, the perfusate was switched to 1 m*M* K^+ + 5.4 m*M* Ca^{2+} in this preparation as well. We thus studied induction of DAD and TA during perfusion with the low K^+ + high Ca^{2+} solution in 24 preparations, excluding the preparation in which DAD developed in the K^+-free perfusate. Twenty of the 24 preparations (83%) developed DAD, with one preparation further developing TA. Therefore, the inducibility of DAD and TA was significantly higher in 1 m*M* K^+ + 5.4 m*M* Ca^{2+} perfusate than in K^+-free perfusate ($P < 0.001$).

Role of the cycle length and number of stimuli in inducing DAD and TA

DAD and TA were never produced by stimulation at the two longer cycle lengths of 1000 ms and 600 ms, being produced only at the shortest cycle length of 300 ms. At the cycle length of 300 ms, a train of 10 stimuli induced DAD in 6 preparations, whereas that of 20 stimuli induced DAD in 22 preparations (Table 1). The difference in inducibility of DAD and TA between the shorter and longer trains was statistically significant ($P < 0.001$).

Correlation of the number of stimuli with the coupling interval and amplitude of DAD and TA

In 6 preparations in which both trains of 10 and 20 stimuli at the cycle length of 300 ms induced DAD or TA, the coupling interval and amplitude of DAD and/or TA were analyzed. The coupling interval was measured between the upstroke of the preceding action potential and the peak of DAD or the upstroke of TA. The amplitude was measured from the maximum negative membrane potential immediately preceding the DAD to the peak of the DAD. Since the trains of stimuli were applied three times during each test perfusion (5, 10, and 20 min after the start of perfusion), a total of 18 trials were made for the trains of 10 and 20

Table 2. Correlation of the number of stimuli with the coupling interval and amplitude of DAD and TA

Cycle length and number of stimuli	Coupling interval of DAD or TA (ms) $n = 16$	Amplitude of DAD (mV) $n = 14$
300 ms, 10	970 ± 182	5 ± 4
	*	**
300 ms, 20	878 ± 186	7 ± 4

Values are means ± SD. *, $P < 0.05$; **, $P < 0.02$

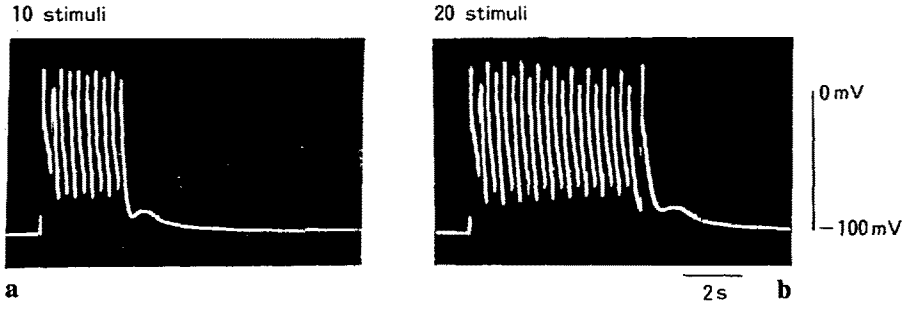

Fig. 1 a, b. Development of delayed afterdepolarization and triggered activity in a rabbit ventricular muscle preparation superfused with 1 m*M* K^+ + 5.4 m*M* Ca^{2+} solution. The preparation was stimulated with trains of 10 and 20 pulses at the cycle length of 300 ms. Note that a triggered action potential and another delayed afterdepolarization were induced after the train of 20 pulses (**b**), whereas only delayed afterdepolarization was induced after 10 pulses (**a**)

pulses in these 6 preparations; 16 of these trials showed the induction of DAD or TA by both trains. The coupling interval of DAD or TA averaged 970 ms with 10 stimuli and 878 ms with 20 stimuli (Table 2), the latter being significantly shorter than the former ($P < 0.05$). In 2 of the 16 positive trials, an immediate development of TA precluded the measurement of DAD amplitude, and hence, the amplitude was compared in 14 trials. The mean DAD amplitude was 5 mV with the train of 10 stimuli and 7 mV with 20 stimuli (Table 2). The difference between these two values was also statistically significant ($P < 0.02$). In the experiment shown in Fig. 1, a train of 10 stimuli produced DAD (**a**), whereas 20 stimuli caused a triggered action potential followed by another DAD (**b**).

Suppression of DAD and TA by mexiletine

The effects of mexiletine on DAD and TA were then studied. The preparation in which TA developed 10 min after the start of the K^+-free perfusion failed to show either DAD or TA after 20 min, so we switched the perfusate to 1 m*M* K^+ + 5.4 m*M* Ca^{2+} solution without testing the effects of mexiletine. Of the 20 preparations in which DAD and/or TA were induced during 1 m*M* K^+ + 5.4 m*M* Ca^{2+} perfusion, one preparation showed DAD only after 10 min and not after 20 min of perfusion, and hence, the effects of mexiletine could not be evaluated. In four other preparations, a stable microelectrode impalement was not maintained after the addition of mexiletine. Thus, a total of 6 preparations were excluded and the results from the remaining 16 preparations were summarized in Table 3. In 15 of the 16 preparations (93.8%), DAD and TA were suppressed by mexiletine. Of these 15, seven preparations (47%) maintained a 1 : 1 response to the electrical stimuli (Fig. 2, *top center panel*), whereas in 8 preparations (53%) the disappearance of DAD and TA was associated with a failure of 1 : 1 response during the rapid stimulation (Fig. 2, *bottom center panel*).

In order to prove that the disappearance of DAD and TA was indeed caused by mexiletine, we tested the effects of wash-out of the drug in 5 preparations. Within 30 min after starting the wash-out of the drug, DAD recurred in 3 of the 5 preparations (Fig. 2, *right panels*). In another preparation in which only DAD was present before the addition of mexiletine, wash-out of the drug was followed by the development of TA. The remaining preparation, which showed a failure of 1 : 1 response to the rapid stimuli during mexiletine perfusion, now responded in a 1 : 1 fashion after wash-out of the drug, but DAD or TA did not recur. The mean values of DAD amplitude before mexiletine perfusion and after its wash-out were 7 mV and 4 mV, respectively ($n = 3$). Similarly, the coupling intervals of DAD before mexiletine and after wash-out were 837 ms and 1064 ms, respectively ($n = 4$). Thus, the amplitude

Table 3. Effects of mexiletine on DAD and TA

	No. of preparations	Suppression of DAD or TA
K^+-free perfusate	1	1 (100%)
1 m*M* K^+ + 5.4 m*M* Ca^{2+} perfusate	15	14 (93.3 %)
Total	16	15 (93.8%)

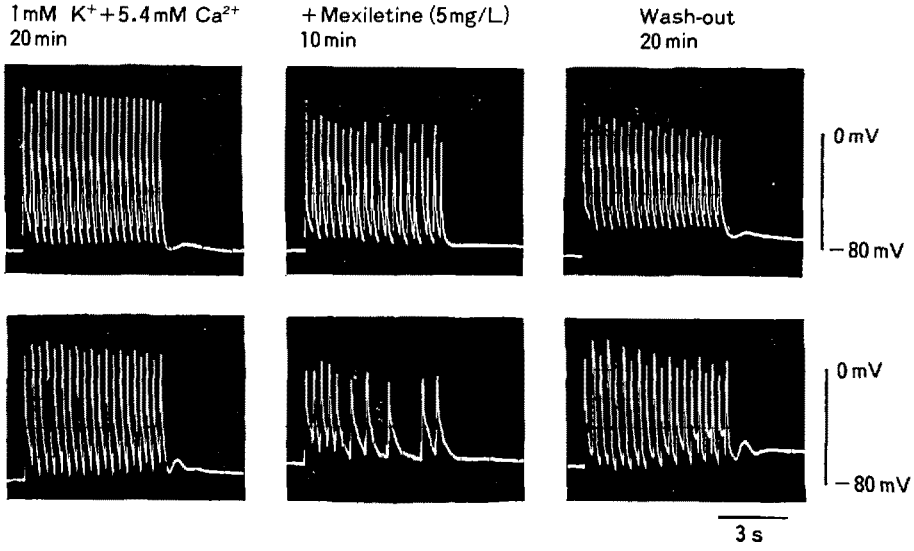

Fig. 2. Suppression of delayed afterdepolarization by mexiletine in two different preparations. In the *top panels*, DAD was abolished in the presence of a 1 : 1 response to the electrical stimuli, whereas in the *bottom panels*, disappearance of DAD was associated with a failure of 1 : 1 response. Wash-out of mexiletine restored delayed afterdepolarization in both preparations

tended to be smaller and the coupling interval longer after wash-out of the drug as compared to the control values, but the differences were not statistically significant ($P > 0.1$). These observations indicate that the suppression of DAD and TA was indeed caused by mexiletine.

Time course of suppression of DAD and TA

In these 16 preparations, DAD or TA was induced 22 times before the addition of mexiletine (6 times by trains of 10 stimuli at the cycle length of 300 ms, and 16 times by trains of 20 stimuli at this cycle length). Five minutes after the addition of mexiletine, DAD was successfully induced in 14 trials, or 8 trials (36%) fewer than the 22 before mexiletine perfusion. Similarly, 10 min after mexiletine, the induction of DAD was observed 6 times (16 less than control, or a decrease of 73%); 20 min after mexiletine, DAD was induced only twice, or 20 times (91%) less than control. In one preparation only, DAD was not suppressed by mexiletine.

Effects of mexiletine on the coupling interval and the amplitude of DAD

The effects of mexiletine on the coupling interval and amplitude of DAD were analyzed by comparing their mean values before and after the addition of the drug in 14 trials in which DAD persisted after the addition of the drug. As the coupling interval was prolonged and the amplitude was reduced progressively after the addition of the drug, we used the longest coupling interval and the minimum amplitude of DAD observed prior to their abolition, to evaluate the drug effects. The amplitude was considered to be 0 mV when DAD could not be produced after the addition of mexiletine. The mean values of the coupling interval before and after the addition of the drug were 969 ± 63 ms and 994 ± 83ms, respectively. Mexiletine thus tended to prolong the coupling interval, but the difference was not significant ($P > 0.1$, $n = 14$). On the other hand, the mean values of the amplitude before and after the addition of the drug were 4 ± 2 mV and 1 ± 4 mV, respectively. The reduction in the amplitude of DAD caused by mexiletine was statistically significant ($P < 0.001$, $n = 14$).

Discussion

In the present experiment, DAD and TA never developed under control conditions, in modified Tyrode's solution. In a comparison of the two test perfusates, DAD was seldom induced in the K^+-

free solution (8%), whereas it was much more readily produced in the presence of 1 m*M* K^+ + 5.4 m*M* Ca^{2+} (83%). Furthermore, the incidence of TA was much lower (4%) than that of DAD, irrespective of the type of solution. Based on these results and many earlier studies, arrhythmias dependent on TA are considered to occur only under certain abnormal conditions such as myocardial ischemia [2, 4, 16, 17], cardiac glycoside intoxication [3–6], catecholamine administration [7, 8], and elevation of intracellular Ca^{2+} concentration induced by electrolyte imbalance [8, 10]. It is known that DAD is caused by a transient inward current and that this current is dependent on a phasic release of Ca^{2+} from (and its reuptake into) the sarcoplasmic reticulum in the presence of high intracellular Ca^{2+} concentrations. The greater ease of DAD induction with the low K^+ + high Ca^{2+} perfusion than with the K^+-free perfusion in the present study may suggest the attainment of a higher intracellular Ca^{2+} level in the former solution.

Our present results—that DAD and TA were induced only by stimulation at the shortest cycle length of 300 ms and that the amplitude of DAD was higher after the train of 20 pulses than after 10 pulses—are consistent with other reports [2, 3, 7, 9].

These observations suggest that DAD and TA seldom develop in the presence of normal sinus rhythm, and hence, the possibility of their playing a major role in the genesis of premature beats and various tachyarrhythmias may well be questioned. However, several of the aforementioned conditions facilitating DAD and TA may sometimes coexist in the clinical setting. In myocardial infarction, for instance, ischemia and intracellular Ca^{2+} loading would occur, and a release of catecholamines from nerve endings is also expected. Under such conditions, the possible role of DAD and TA in causing ventricular tachyarrhythmias should not be underestimated. Regarding the effects of mexiletine, this drug suppressed DAD and TA in 94% of the preparations (Table 3) at the concentration of 5 mg/l [18]. Rosen and Danilo reported the effects of lidocaine, another class 1B antiarrhythmic drug, on DAD induced by ouabain [19]. Watanabe reported that disopyramide, a class 1A antiarrhythmic drug, also suppressed DAD and TA induced by high concentrations of lanatoside C [20], and Wasserstrom and Ferrier showed the effects of quinidine on oscillatory afterpotentials induced by acetylstrophanthidin [21]. Therefore, such an action appears common to most class 1 agents.

Possible electrophysiologic mechanisms of class 1 antiarrhythmic drugs in suppressing DAD and TA must now be discussed. First, it is well known that class 1 antiarrhythmic drugs block both the fast Na^+ current during action potential phase 0 and the Na^+ current in the steady state [22, 23]. Eisner et al. reported that lidocaine decreased the magnitude of the transient inward current (I_{TI}). They reasoned that lidocaine reduced intracellular Na^+ activity, and this, in turn, decreased intracellular Ca^{2+} concentration [24]. Kass et al., using sheep Purkinje fibers [13], showed that tetrodotoxin suppressed TA when the fibers failed to respond to the electrical stimuli in a 1 : 1 fashion. These authors thus assumed that the suppression of TA by tetrodotoxin was secondary to the reduction of the number of action potentials leading to the lowering of intracellular Ca^{2+} concentration. Since, in our present study, suppression of DAD and TA by mexiletine was preceded by a failure of 1 : 1 response in 8 of the 15 preparations (53%), it could be suggested that the aforementioned mechanism at least partially contributed to the efficacy of this class 1B drug. However, in the remaining 7 preparations (47%), DAD and TA were suppressed in the presence of a 1 : 1 response to the electrical stimuli, and therefore, certain other mechanisms may have to be considered as discussed below.

In our voltage clamp experiments using small preparations of the rabbit atrioventricular node, disopyramide has been shown to decrease the slow inward current [20]. Ono et al. reported that mexiletine and lidocaine blocked the Ca^{2+} current in isolated ventricular myocytes [25]. These class 1 drugs may thus be said to act like class 4 antiarrhythmic drugs or Ca^{2+} antagonists. Blockage of the slow inward current would lower intracellular Ca^{2+} concentration and secondarily decrease the transient inward current. Suppression of DAD and TA by disopyramide, mexiletine, or lidocaine may be explained on this basis. On the other hand, a group of investigators demonstrated that a prolongation of the action potential duration tended to facilitate the development of DAD and TA, and a shortening of the action potential duration had the opposite effect [26]. Thus, it may be argued that class 1B antiarrhythmic drugs such as mexiletine could suppress DAD and TA simply by shortening the action potential duration.

Still other mechanisms by which class 1 antiarrhythmic drugs could suppress TA have been suggested [27]. Since class 1 antiarrhythmic drugs are known to depress both excitability and conductivity in myocardial cells, they may either prevent the generation of an action potential (TA) even when DAD is present, or may block the conduction of TA to the neighboring myocardium, thus preventing premature systoles and other

tachyarrhythmias. However, the possible role of these mechanisms cannot be assessed by the present study.

Finally, possible clinical implications of the present results will be briefly discussed. Since mexiletine was effective in suppressing DAD and TA during either K^+-free or low K^+ + high Ca^{2+} perfusion and also in the presence of ouabain [28, 29], this drug may well be indicated against ventricular tachyarrhythmias occurring under similar conditions. On the other hand, it has sometimes been proposed that successful termination of a given tachyarrhythmia by a class 1 antiarrhythmic drug probably suggests a re-entry mechanism rather than triggered activity. This notion certainly cannot be validated in view of the present observations, and attempts to identify the electrophysiologic mechanisms of clinical arrhythmias from their responses to different antiarrhythmic drugs do not appear promising, at least in ventricular tachyarrhythmias.

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