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Effect of Urapidil on The Action Potentials in The Guinea-Pig Ventricular Myocardium

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Summary. **The electrophysiological effects of urapidil, a new α_1-adrenoceptor antagonist, were assessed in the reserpinized guinea-pig ventricular myocardium. Urapidil suppressed the maximal rate of rise ($\dot{V}_{max}$) of steady-state action potentials elicited by the fast responses at high concentrations independently of blockade of myocardial α-adrenoceptors, but not the $\dot{V}_{max}$ of Ca^{2+}-dependent slow action potentials of partially depolarized muscles in concentrations tested (up to 1.1 mM). Urapidil at high concentrations prolonged the action potential durations of the fast and slow responses in a manner similar to the quinidine-like antiarrhythmic drugs. These results suggest that the inhibitory effect of urapidil on the slow inward Ca^{2+} current and the Na^+ current is in practice negligible.**

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Key Worlds. **urapidil, α-blocker, depolarized muscle, slow action potential, sodium channel, calcium channel**

Several α_1- and α_2-adrenoceptor antagonists have demonstrated their suppressive effect on various experimental arrhythmias [1–5]. Northover [6] considered the antiarrhythmic effect of prazosin, yohimbine, tolazoline, dibenamine, and phenoxybenzamine on the interaction with cell membranes and not on the inhibition of the α-adrenoceptors. Hasegawa et al. [7] reported the inhibitory effects of α-adrenoceptor antagonists on the transient depolarization and the triggered activity and protective effect on the worsening of abnormal automaticity induced by hypoxia. They also suggest that α-adrenoceptor antagonists exert not only their α-adrenoceptor antagonistic effect, but also the fast Na^+ and slow calcium-channel blocking effect in a manner similar to antiarrhythmic drugs with slow kinetics. In this respect, the electrophysiological effects of several α-adrenoceptor antagonists have been studied [8–11]. On the other hand, antiarrhythmic drugs occasionally show a proarrhythmic effect in clinical use due to the same electrophysiological properties (such as inhibitory effects of membrane currents) as the antiarrhythmic effect on the myocardium. Urapidil is a new α_1-adrenoceptor antagonist and is used for its vasodilator action in patients with hypertension. Urapidil has been reported to have an inhibitory effect on the fast response of ventricular myocardium [12] and the pacemaker activity of the sinoatrial node [13].

The present experiments were carried out to determine whether urapidil suppresses the Ca^{2+}-dependent slow response, as well as the fast response in guinea-pig cardiac ventricular muscle, as if it does, whether each inhibitory effect is significant.

Materials and Methods

Guinea-pig papillary muscles were rapidly excised from the right ventricle and mounted in a tissue bath perfused with oxygenated (95% O_2, 5% CO_2) Tyrode solution (pH 7.4 ± 0.05). The bath temperature was continuously monitored with an electric thermometer and maintained at 36.0 ± 0.2°C. The muscles were stimulated at a rate of 1 Hz (voltage 1.5 times threshold, duration 1 ms) through bipolar Ag-AgCl electrodes connected to a stimulator (Nihon Kohden SEN-3201) throughout the experiment. Membrane potentials were measured differentially using two microelectrodes filled with 3 M KCl (resistance 15–20 MΩ) and fed into an amplifier (Nihon Kohden MEZ-7101). Analog differentiation of the action potential was obtained with an electric differentiator that was linear from 0 to 500 V/sec (time constant 10 μs usually, and 50 μs for slow action potentials). The action potential and the maximum rate of rise ($\dot{V}_{max}$) of the action potential were monitored on an oscilloscope (Nihon Kohden VC-10), photographed by a camera (Nihon Kohden RLG-6201), and recorded simultaneously on a recticorder (Nihon Kohden RJG-4122).

Normal Tyrode solution had the following composition (in mM): NaCl 132, KCl 4, $NaHCO_3$ 12, NaH_2PO_4 0.4, $CaCl_2$ 1.8, $MgCl_2$ 1, and glucose 10. The high K^+ solution was prepared by increasing the concentration

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of KCl to 27 mM and by adding 0.2 mM $BaCl_2$ without adjusting osmolarity to elicit the Ca^{2+}-dependent slow response without the application of catecholamine [14,15]. To avoid the interference of endogenous norepinephrine, the catecholamine content in the heart was lowered by pretreating the guinea pigs with 2.5 mg/kg reserpine (Daiichi) subcutaneously, 1 day before they were killed.

All experimental results were obtained from a single continuous impalement throughout the experiment. Values are given as mean ± SEM. Statistical analysis was performed by Student's t test. The results were considered significant when p was <0.05.

Results

Effects of Urapidil on Steady-State Action Potentials in Normal Tyrode Solution

Figure 1 shows the inhibitory effects of urapidil on the action potentials of fast responses. Urapidil decreased $\dot{V}_{max}$ of action potentials and prolonged the action potential duration at the 90% repolarization level (APD) from the control values of 192.7 ± 14.8 V/sec for $\dot{V}_{max}$ and 235.0 ± 9.8 msec for APD, to 139.2 ± 17.2 V/sec ($P < 0.05$) and 280.3 ± 13.9 msec ($p < 0.005$), respectively, at a rather high concentration of 0.3 mM urapidil. The action potential amplitude (APA, 128.5 ± 1.5 mV in the control) and the resting potential (92.5 ± 1.4 mV in the control) was not influenced by urapidil.

Effects of Urapidil on the Ca^{2+}-Dependent Slow Action Potentials Stimulated at 1 Hz

Figure 2 shows the effects of urapidil on the Ca^{2+}-dependent slow action potentials in partially depolarized cardiac muscle in the high K^+ solution. Urapidil prolonged the action potential duration in a dose-related manner, but did not change $\dot{V}_{max}$, the resting potential, and the amplitude of action potentials, even at its

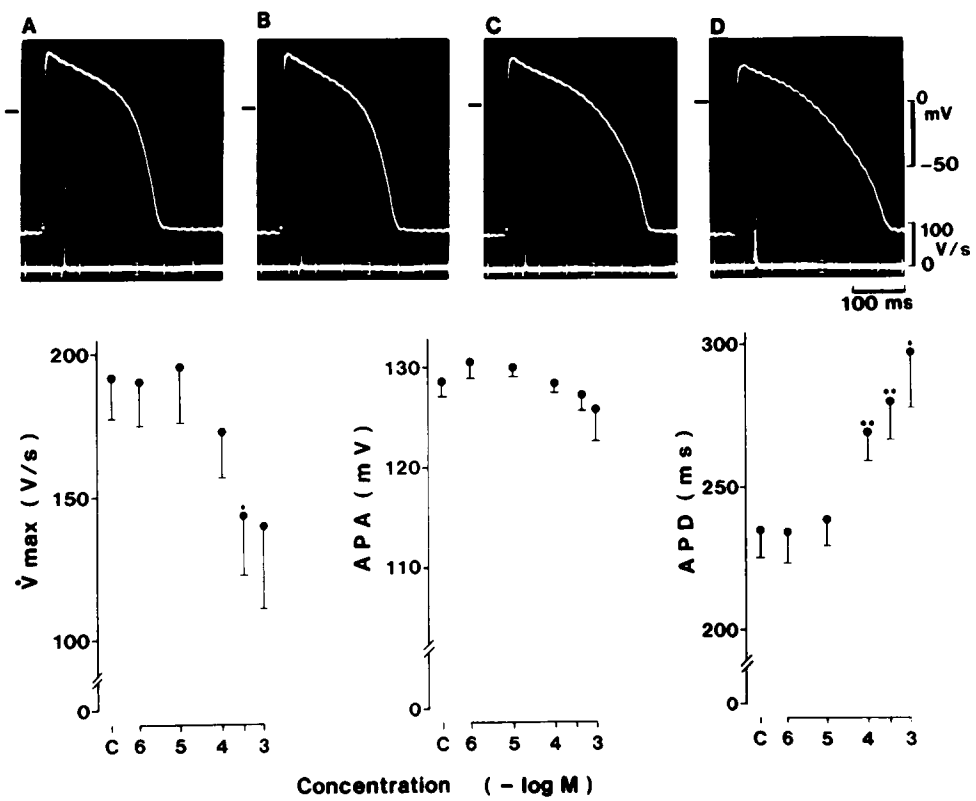

*Fig. 1. Depressant effect of urapidil on the fast responses. Upper panel shows the action potentials stimulated at a constant rate of 1 Hz and their maximal rate of rise ($\dot{V}_{max}$) in normal Tyrode (A), in the presence of 10^{-5} (B), 10^{-4} (C), and 10^{-3} M (D) urapidil. In this and the following figure, the short horizontal lines preceding the action potential traces indicate the 0 mV level. Dose-response relationships of the effects of urapidil on $\dot{V}_{max}$, action potential amplitude (APA), and action potential duration at the 90% repolarization level (APD) are shown in the lower graphs. Each point represents the mean value, and vertical bars are the SEM of six experiments. Mean values of $\dot{V}_{max}$, APA, and APD in the control conditions for six experiments were 192.7 V/sec, 128.5 mV, and 235.0 msec, respectively. * $p < 0.05$; ** $p < 0.01$.*

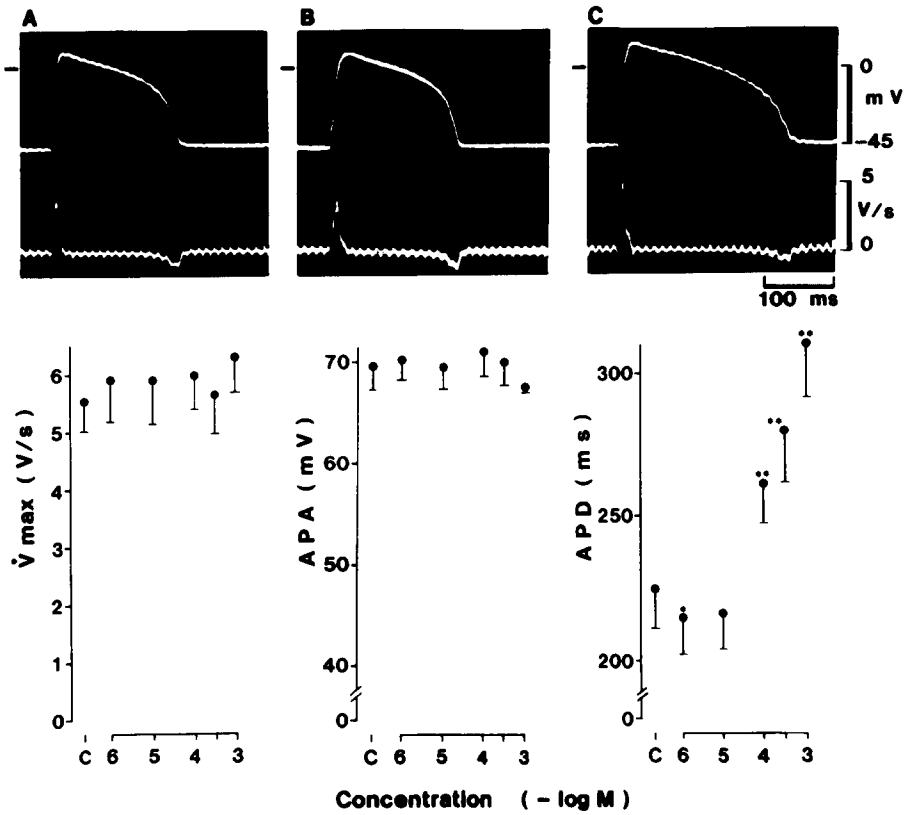

Fig. 2. *Effects of urapidil on the Ca^{2+}-dependent slow action potentials. Upper panels show action potentials of partially depolarized muscle and their $\dot{V}_{max}$ in control (A) and in the presence of 10^{-5} (B) and 10^{-3} (C) M urapidil. The lower graphs show the dose-response relationships for the effects of urapidil on the parameters of the slow action potentials. The abbreviations are as in Figure 1. The mean values of $\dot{V}_{max}$, APA, and APD in the control conditions for six experiments were 5.5 V/sec, 69.5 mV, and 225.6 msec, respectively.*

high concentrations, from control values of 225.6 ± 13.9 msec for APD, 5.5 ± 0.5 V/sec for $\dot{V}_{max}$, 47.0 ± 2.2 mV for the resting potential, and 69.5 ± 2.3 mV for APA, respectively.

Discussion

Several α-adrenoceptor antagonists have been shown to have an inhibitory effect on the generation of action potentials, in addition to their antagonistic effect on α-adrenoceptors. This extra-adrenergic effect of drugs may be derived from their inhibitory action on the fast Na^+ and slow inward Ca^{2+} current of the cell membrane. Hasegawa et al. [7] demonstrated the role of inhibitory effects on the membrane currents, as well as the α-adrenoceptor blocking effect in the suppression of experimental arrhythmias. Although Kotake et al. [13] reported an inhibitory effect on the slow inward Ca^{2+} current in rabbit sinoatrial node preparations, such an effect of urapidil on ventricular muscle has not been well substantiated. Gulch et al. [12] showed the effect of urapidil on action potentials in normal Tyrode solution only at concentrations higher than 10^{-3} M. They speculated that it had an effect on the slow inward Ca^{2+} current because of its suppression of contactile force. Since contractile force of muscle can be influenced not only by the Ca^{2+} current, but also by some other mechanisms that affect the stored Ca^{2+} [16], such as the Na^+ current inhibition through Na^+-Ca^{2+} exchange, and Na^+-K^+ ATPase inhibition [17–19], the information on muscle contraction seems to provide insufficient evidence for suppression of the Ca^{2+} current.

In the present study, $\dot{V}_{max}$ of the fast and slow action potentials were measured as indices of the fast Na^+ and slow Ca^{2+} currents of the membrane, respectively, instead of via direct measurements. Recently, a nonlinear relation between Na^+ current and $\dot{V}_{max}$ has been reported [20]; however, the usefulness of $\dot{V}_{max}$ measurement as a simple and rough index of the

peak inward Na^+ current does remain. Although these limitations are also expected in the relation between $\dot{V}_{max}$ of the slow action potential and the slow inward Ca^{2+} current, the most important results of the present study may not be diminished by these limitations. The concentrations of urapidil effective on the Ca^{2+}-dependent slow action potentials and the fast responses were examined in reserpinized preparations, and it was revealed that urapidil inhibited the fast Na^+ current (as assessed by $\dot{V}_{max}$) but not the slow inward Ca^{2+} current, even using high concentrations of drug in ventricular myocardium. Although the effect of urapidil on the action potential duration resembles those of quinidine-like antiarrhythmic drugs and may be ascribed to inhibition of the inward rectifier K^+ current and the delayed outward current, this effect appears only at rather high concentrations. Urapidil concentrations of 0.7 to 2.0 μM are found after intravenous administration [21] and of 0.2 to 2.6 μM after a single dose oral administration [21]. Similar concentrations in our study showed no remarkable changes in the electrophysiological properties of the ventricular myocardium.

Since the present experiments were performed on the papillary muscles of guinea pigs pretreated with reserpine without the application of catecholamines, even for the induction of the slow responses, the inhibitory effects on the membrane currents by urapidil might be independent of the blockade of cardiac α-adrenoceptors.

These results suggest that the effects of urapidil on the fast Na^+ and slow inward Ca^{2+} currents are rather negligible in concentrations that are similar to those found clinically.

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