Effects of chlorobutanol and bradykinin on myocardial excitation

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Hermseyer, Kent and Octavio Aprigliano. Effects of chlorobutanol and bradykinin on myocardial excitation. Am. J. Physiol. 230(2): 306-310. 1976. — The negative inotropic effect of a commonly used formulation of bradykinin (Sandoz BRS-640) was found to be due to chlorobutanol, a constituent of the preparation. Solutions containing up to 100 μg of crystalline bradykinin/ml had no effect on tension or action-potential shape. Chlorobutanol (600 μg/ml) caused a 30% decrease in contraction amplitude and a 20% increase in action-potential duration. Chlorobutanol lowered conduction velocity and induced conduction failure and automaticity within isolated ventricular muscle strips. Chlorobutanol affected neither positive nor negative treppe. We conclude that bradykinin has no direct action on toad, frog, or rat myocardium. However, chlorobutanol does have direct effects on myocardial cells, acting on the cell membrane and decreasing isometric tension produced by the heart.

The effects of bradykinin on nonstriated muscles have been well demonstrated (16). However, there is no conclusive evidence for a direct action of the polypeptide on cardiac muscle. Bishop et al. (4) and Montague et al. (12) found positive inotropic effects of bradykinin injected systemically. Nakano (14) observed a decrease in contractility of isolated guinea pig atria in response to bradykinin on contraction of isolated frog and Mercuraria hearts. The mechanism for the reported effects has not been determined and indeed the apparent contradiction has not been resolved or even confirmed. The bradykinin solutions used for the in vitro experiments were supplied by Sandoz Pharmaceuticals and are characteristic chlorobutanol-containing solutions. There have been several reports that chlorobutanol decreases development of tension by muscle cells (Axelsson et al., 1), Somlyo et al. (19), Woo and Somlyo (20), Somlyo and Somlyo (18), Belither (3), and Ayers et al. (2). Epinephrine and several peptides are commonly supplied with chlorobutanol as a preservative (antineptic) in concentrations high enough to significantly affect contraction. In the course of looking for the mechanism of the negative inotropic effect of bradykinin on isolated toad myocardium, we noted that the effect was found in that formulation supplied by Sandoz Pharmaceuticals (BRS-640), but not when solutions made from pure crystalline bradykinin were used. The experiments described showed that the direct negative effect of Sandoz bradykinin on the myocardium was due to the chlorobutanol. Since the mechanism of action of chlorobutanol has received limited attention, we also studied the membrane-dependent changes and looked for evidence of an action on the excitation-contraction coupling mechanisms involved in treppe.

The present report thus has two main purposes: 1) to demonstrate that bradykinin has no direct action on the myocardium, an assertion that does not appear to have been made in the literature; and 2) to show that what has been reported to be due to bradykinin is explicable by the actions of the adjuvant chlorobutanol, which exerts a direct membrane excitation action and depressed contractility.

Methods

The hearts of 22 toads (Bufo marinus) and 6 frogs (Rana pipiens) were excised, after the animals were pithed and placed in amphibian Ringer solution composed of (in mM): 114 Na⁺, 1.88 K⁺, 0.92 Ca²⁺, 115.84 Cl⁻, 1.88 HCO₃⁻, and 5.5 glucose. A ventricle strip was dissected, mounted in a flow-through muscle bath (6 ml), and suffused with amphibian Ringer solution gassed with 95% O₂ and 5% CO₂ (pH 6.8-7.2) at room temperature (20-25°C). Hearts and duodenal segments were removed from six rats anesthetized with pentobarbital and 3-mm-wide strips of the right ventricle were cut. The rat muscles were kept in continuously oxygenated electrolyte solution (pH 7.4) composed of (in mM): 149.3 Na⁺, 4.7 K⁺, 1.8 Ca²⁺, 0.4 Mg²⁺, 142.1 Cl⁻, 16.3 HCO₃⁻, and 7.8 glucose. The myocardial strips were placed under 2 kgf of force and duodenal segments were placed under 500 dyn of force and continuously suffused (without recirculation) at 37°C in 5-ml chambers.

Myocardial cells were impaled with glass microelectrode electrodes, filled with 3 M KCl and having a resistance of 15-30 MΩ, by a floating microelectrode technique: Ag-AgCl half-cells provided electrolyte-amplifier connections. The action potentials were displayed on a Tektronix oscilloscope through an electrometer with input impedance of > 10¹⁴ Ω and rise time of < 10 μs. The rate of rise of the action potential was derived...
by an operational amplifier differentiator, the output of which was displayed on the second oscilloscope beam. Conduction velocity was determined by two electrodes at a measured interval. Mechanical recordings were made with a Grass force-displacement transducer, coupled to a Grass polygraph. A Grass stimulator delivered 5 ms square pulses at the specified rates through a stimulus isolation unit and Pt pin electrodes.

The following drug solutions were used. Bradykinin BRS-640 (kindly supplied by Sandoz Pharmaceuticals), containing 0.1 mg bradykinin, 5 mg glacial acetic acid, 2 mg sodium acetate·3H2O, 5 mg chlorobutanol, 7 mg sodium chloride, and 1.005 g distilled water, was diluted in the appropriate physiological salt solution. Solid forms of bradykinin (Sigma Chemical Co.), chlorobutanol (Merck Sharp & Dohme), acetylcholine chloride (Calbiochem), atropine sulfate (Sigma), norepinephrine (Sigma), and phentolamine (Ciba Pharmaceutical Company) were measured and added directly to the physiological salt solution immediately before use.

RESULTS

Lack of effect of bradykinin on cardiac contraction. The effects of bradykinin, BRS-640, and chlorobutanol solutions on isometric tension developed by toad ventricular myocardium are shown in Fig. 1. Frog and rat ventricles showed similar reductions in isometric tension in BRS-640 and chlorobutanol solutions. The decrease in tension produced by BRS-640 was indistinguishable from that produced by the equivalent concentration of chlorobutanol in the bathing solution. On the other hand, a bradykinin concentration equivalent to that in the BRS-640 produced no change in isometric tension. Even 100 µg of bradykinin/ml never produced any change in isometric tension in any of the three species in these experiments. However, solutions of the same bradykinin at a concentration of 1 ng/ml produced marked relaxation of the spontaneously contracting isolated rat duodenum within 30 s. Duodenal spontaneous contraction frequency before bradykinin was 15/min. After 1 ng of bradykinin/ml, contractions completely disappeared at 30 s and resumed 1 min or more after bradykinin was washed out. The decrease in myocardial tension produced by chlorobutanol was rapid in onset, appearing within 15 s and reaching a steady state within 5–10 min; amplitude of the isometric contractions remained depressed during continued perfusion with chlorobutanol solution and returned to about control level within 10 min after chlorobutanol washout.

Effect of chlorobutanol on cardiac action potential. The prolongation of the cardiac action potential by chlorobutanol is shown in Fig. 2A. Chlorobutanol caused a marked increase in the length of the plateau that began within 15 s and reached a nearly steady state in 5–10 min in toad ventricle. Frog ventricle showed similar action-potential prolongation, but rat ventricle showed only action-potential shortening. Lengthening of the action potential gradually continued to maximal durations at about 50 min. The actions of chlorobutanol were not altered by either atropine (35 µM) or phentolamine (10 µM), which completely blocked the responses to 10 µM acetylcholine or 1 µM norepinephrine, respectively. No noticeable change in resting $E_m$ (−85 mV) or amplitude of the action potential plateau occurred as a result of the chlorobutanol. After 1 h in 500 µg of chlorobutanol/ml, stimulation of toad ventricle at 48/min sometimes induced a marked change in action-potential shape. The spikes were slowly rising with no plateau and occurred with a rate independent of the stimulating pulses (Fig. 2, B and C). These spikes had maximum rates of rise ($+V_{max}$) of only 2–5 V/s, arose from resting membrane potentials of 60 to −80 mV, reached 0 to +15 mV at their peaks, and were 500 ms total duration. After 2–5 min of spontaneous activity these cells would become quiescent, and they again became possible to stimulate at pacing rates lower than 48/min to produce action potentials with full plateaus and higher $+V_{max}$.

The increase in action-potential duration produced by

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**FIG. 1.** Isometric tension of electrically paced toad ventricular myocardium is decreased equally by chlorobutanol and BRS-640 containing an equal chlorobutanol concentration, in contrast to lack of effect of solutions containing bradykinin but not chlorobutanol. Stimulus rate was 24/min, vertical bars are SE, and numbers are at each point. Abarissa is a double scale for bradykinin and chlorobutanol concentration, with ratio between concentrations matching that found in BRS-640. Chlorobutanol: bradykinin weight ratio of 50 is equivalent to a molar ratio of 299.

**FIG. 2.** Actions of chlorobutanol on cardiac action potential. A: superimposed action potentials recorded from toad ventricle paced at 48/min before (a) and 19 min after (b) addition of 500 µg of chlorobutanol/ml. Note prolongation of action potential by chlorobutanol. B, and C: another toad ventricle was exposed to 500 µg of chlorobutanol/ml for 54 min and driven at 48/min until it showed spontaneous spikes with no plateau and lower maximum rates of rise. Horizontal calibration is 400 ms for A and B and 4 s for C. Vertical calibration is 40 mV.
Chlorobutanol occurred at all stimulus rates used, as is shown by Fig. 3. As stimulus rate increased, action-potential duration (measured at full repolarization) decreased in control and chlorobutanol solutions. The relationship between duration and pacing rate was shifted to longer durations by chlorobutanol without significantly altering the slope of the line. The action of BRS-640 on action-potential duration was identical to that of the equivalent chlorobutanol concentration. Bradykinin at concentrations up to 100 pg/ml caused no change of action-potential duration, shape, or \( +V_{\text{max}} \) at any stimulus rate.

**Treppe phenomena.** The toad ventricle showed both positive (Bowditch) and negative (Woodworth) treppe phenomena very prominently. Figure 4A shows that when pacing rate was increased there was an initial increase in isometric contraction amplitude (positive treppe) followed by a slower developing decrease in isometric tension (negative treppe). The reverse occurred on slowing the pacing rate—the positive treppe appeared as a decrease in contraction amplitude and the negative treppe subsequently appeared as an increase in isometric force. Both phenomena can be seen each time the stimulus rate was changed in the toad ventricle at least over the stimulus range from 3 to 48/min. Rat ventricle showed mainly the negative treppe, and frog ventricle showed only positive treppe. In rat and frog ventricles, negative treppe was the initial transient event and positive treppe followed later to produce the steady-state contraction and amplitude, i.e., the time courses were just reversed from those in toad ventricle. After treatment with chlorobutanol, the magnitude of all isometric contractions was less, but both positive and negative treppe phenomena were still apparent (Fig. 4B). No instance of the disappearance of treppe phenomena after chlorobutanol treatment was observed at any pacing rate used in any of the three species.

Conduction disturbances produced in toad myocardium by chlorobutanol. In addition to prolonging the cardiac action potential, chlorobutanol altered conduction of excitation in isolated strips of toad cardiac muscle. Conduction failure along the strip was frequently observed at the 500 \( \mu \)g/ml concentration. In several instances, the excitation pathway was toward the point of stimulation as determined by the order of appearance of action potentials in the two cells impaled by the recording electrodes, even though the cells followed the pacing frequency. In other experiments ectopic foci of pacemaker activity formed, making muscle strips more difficult to pace (i.e., stimulating current was increased to maintain rate control or rate could not be controlled in some ventricle strips). During this time, action potentials were still of increased duration. Refractory period increased by about 200 ms, as is shown in Table 1. The maximum rate of rise of the action potential \( (+V_{\text{max}}) \) was decreased by about 40%, and conduction velocity \( (\theta) \) decreased from 5.0 cm/s to 2.4 cm/s. Increasing the stimulus rate caused more extra spikes to occur without detectably altering the \( +V_{\text{max}} \) except during the pronounced change in action potential form noted above (Fig. 2) when stimulus rate was increased to 48/min.

**DISCUSSION**

The present experiments show that bradykinin has no immediately observable action on isolated ventricular myocardium action potential parameters

**TABLE 1. Actions of chlorobutanol on toad ventricular myocardium action potential parameters**

<table>
<thead>
<tr>
<th>Test</th>
<th>Maximum Rate of Rise, ( V_{\text{Na}} )</th>
<th>Conduction Velocity, cm/s</th>
<th>Refractory Period, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>( 29 \pm 3 ) ( \pm 5.0 \pm 0.3 )</td>
<td>( 960 \pm 20 )</td>
<td>( n = 56 ) ( n = 8 ) ( n = 27 )</td>
</tr>
<tr>
<td>Chlorobutanol</td>
<td>( 18 \pm 4 ) ( 2.4 \pm 1.0 )</td>
<td>( 1150 \pm 100 )</td>
<td>( n = 21 ) ( n = 6 ) ( n = 9 )</td>
</tr>
</tbody>
</table>

All differences are significant at \( P < 0.05 \) confidence level (t-test comparison). Resting \( E_{\text{Na}} \) was not altered by chlorobutanol. Stimulation rate was 18/min and temperature was 21°C.
The effects of chlorobutanol on contraction do not appear to depend on alterations in excitation-contraction (EC) coupling because the treppe phenomena, which are both prominent in toad ventricle, are unchanged. Treppe phenomena have been proposed by several workers as being due to altered Ca²⁺ availability through EC coupling mechanisms (5, 8, 10, 13, 17). Chlorobutanol decreased the contractile force proportionally whether stimulus rate was constant, increasing, or decreasing. Such a uniform effect on contraction would not be expected if chlorobutanol acted on a specific site in the EC coupling sequence, e.g., altered intracellular Ca²⁺ binding (7). For example, Ca²⁺ removal or exposure to ryanodine was reported to specifically eliminate negative treppe (10). The action of chlorobutanol on membrane excitation would cause a contractile force decrease without alteration of the treppe phenomena because desynchronization of contraction would not be expected to alter specific EC coupling mechanisms. Of course, chlorobutanol may act through mechanisms other than membrane excitation. For example, Campello et al. (6) reported that chlorobutanol caused inhibition of NADH dehydrogenase in liver cells.

These demonstrations that chlorobutanol is a dysrhythmogenic agent are significant for the therapeutic use of epinephrine, which is administered by intracardiac injection in cardiac arrest. Some of the preparations of epinephrine contained 0.5% chlorobutanol, which this report has shown can cause conduction disturbances and decreased contractility. The direct dysrhythmogenic action of epinephrine may well have been enhanced by a separate dysrhythmogenic action of chlorobutanol. The results of the present study would strongly suggest that the use of chlorobutanol in solutions of epinephrine and vasopressin should be critically evaluated.

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