Reversal of Ouabain-Induced Electrophysiological Effects by Potassium Canrenoate in Canine Purkinje Fibers

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ABSTRACT

Canrenoate, an aldosterone antagonist, was administered to normal and ouabain-treated Purkinje fibers excised from the hearts of mongrel dogs and perfused with Tyrode’s solution at 35–37°C. Intra- and extracellular potentials were recorded with conventional techniques. Potassium canrenoate in concentrations up to $1 \times 10^{-3}$M or sodium canrenoate in concentrations up to $5 \times 10^{-4}$M had inconspicuous electrophysiological effects on untreated Purkinje fibers. The duration of the action potential was slightly shortened. Amplitude of resting and action potentials, rate of rise of action potentials, conduction velocity, membrane responsiveness, automaticity, and excitability were not significantly altered by canrenoate. In contrast, potassium canrenoate in concentrations of $1 \times 10^{-6}$M to $5 \times 10^{-4}$M produced rapid restoration toward normal of fibers moderately or severely affected by ouabain ($10^{-7}$M). Resting and action potentials were augmented, rate of rise of action potentials was increased, excitability was restored, and conduction was enhanced. Also ouabain-induced spontaneous rapid firing could be terminated. Twice equimolar doses of KCl were ineffective. The data suggest that canrenoate might be a specific antagonist to the electrophysiological effects of digitals.

KEY WORDS digitalis excitability aldosterone antagonist transmembrane potentials membrane responsiveness spontaneous firing

Potassium canrenoate, a steroid compound and an aldosterone antagonist, is also a versatile antagonist of the toxic electrophysiological effects of digitalis on the heart in anesthetized (1, 2) and awake (3, 4) dogs. It can prevent or abolish diverse manifestations of toxicity such as sinus arrest, atrioventricular block, and ventricular tachycardia. To gain insight into the manner in which this antagonism is accomplished, we monitored certain of the electrophysiological actions of canrenoate on untreated and ouabain-treated canine Purkinje fibers.

Methods

Preparations of Purkinje fibers and ventricular muscle were excised from the hearts of sodium pentobarbital-anesthetized dogs and perfused with a solution of the following millimolar composition: $\text{Na}^+ 151.1$, $\text{K}^+ 4.0$, $\text{Ca}^{2+} 2.0$, $\text{Mg}^{2+} 0.5$, $\text{Cl}^- 132.5$, $\text{HCO}_3^- 24.0$, $\text{HPO}_4^{2-} 1.8$, and dextrose 5.5. The solution was equilibrated with 95% O$_2$-5% CO$_2$ at 35–37°C. The fibers were stimulated with rectangular pulses 2 msec in duration (Tektronix 160 pulse and ramp generators) delivered through a stimulus isolation unit (Bioelectric IS2) to Teflon-coated silver wires in contact with the preparation. For the determination of threshold current, cathodal stimulation was accomplished by removing the anodal wire from the surface of the tissue. Intracellular recordings were made with machine-pulled glass microelectrodes filled by boiling with 3M KCl. These electrodes had a resistance of 10–40 megohms. The first-stage amplifier (Bioelectric NF1) had adjustable negative capacitance and high input impedance ($10^{12}$ ohms). Bipolar electrograms were recorded through stainless steel wires (0.007 inches in diameter) led into differential amplifiers. Action potentials were differentiated electronically with respect to time by a resistance-capacitance differentiating circuit which gave a linear response to the rate of rise (dV/dt) from zero to 1000 v/sec. The differentiator was calibrated using the attenuated ramp voltage from a sweep-generating circuit of an oscilloscope. The calibrating ramp signal was led into the bath continuously through the indifferent electrode. The voltage drop across 1000 ohms in one of the stimulating leads was monitored through a differential amplifier. Recordings were displayed on a dual-beam oscilloscope (Tektronix 565) and photographed (Grass C4N).

Concentrations of canrenoate in the perfusate ranged from $5 \times 10^{-6}$M to $5 \times 10^{-4}$M, and the drug was administered by continuous infusion (usually employed with untreated fibers) or by bolus injection directly into the tissue chamber (usually employed with ouabain-treated fibers). The bath volume was 50 ml, and the rate of superfusion was 7–10 ml/min so that after bolus injection the half time of the decline of the concentration of the drug was about 3–5 minutes. Ouabain was administered by continuous infusion in...
concentrations of $10^{-8}$ to $10^{-6}$ M. The ouabain infusion was continued while potassium canrenoate was administered. Sodium canrenoate was obtained by passing potassium canrenoate through an ion-exchange resin. Assay of potassium in the effluent established that the exchange was more than 95% complete. Sodium canrenoate was less soluble than potassium canrenoate and tended to precipitate in the stock solutions ($5 \times 10^{-2}$ M).

**Results**

The only consistent effect of potassium canrenoate in concentrations less than $10^{-3}$ M was a slight shortening of the duration of the action potential which occurred quite rapidly. This effect is shown in Figure 1. One minute after the addition of canrenoate directly to the bath, the duration of the action potential (100% repolarization) shortened from 450 msec (A) to 415 msec (B). After 3 minutes, a further shortening to 390 msec had occurred (D). This shortening involved predominantly phase 2.

The failure of potassium canrenoate ($5 \times 10^{-4}$ M) to affect the amplitude, overshoot, and $dV/dt$ of the action potential or the conduction velocity is shown in Figure 2. In this fiber a slight increase in maximal $dV/dt$ of the upstroke ($V_{\text{max}}$) occurred (B), but this effect was not a consistent finding. With concentrations of $10^{-3}$ M, a slight decrease in resting potential was observed, but conduction velocity was unimpaired or slightly increased. $V_{\text{max}}$ was not depressed, and the stimulus current amplitude was unchanged.

Five experiments sodium canrenoate was employed to extend the concentration range studied. Figure 4 shows records from an experiment in which sodium canrenoate was infused at a concentration of $2.5 \times 10^{-3}$ M. There was a very slight shortening of the action potential (predominantly acceleration of phase 2), but other changes were negligible. $V_{\text{max}}$ was not depressed, and the stimulus current amplitude was unchanged.
Automatically was not consistently affected by potassium canrenoate in concentrations up to $10^{-6}$M. Neither the rate of phase-4 depolarization nor the threshold potential of spontaneous pacemaker cells was altered.

In four preparations, the $V_{\text{max}}$ of action potentials generated during repolarization and electrical diastole (phase 4) was measured and plotted against membrane potential at the time of excitation. The resultant "membrane-responsiveness" curve was not significantly shifted by potassium canrenoate in concentrations up to $5 \times 10^{-4}$M (Fig. 5). The strength-interval curve was shifted along the abscissa in proportion to the shortening of the action potential duration, but it was not shifted along the ordinate. Threshold current was plotted against the membrane potential at the time of stimulation. This relationship was not affected by canrenoate, as illustrated in Figure 6.

In contrast to its inconspicuous effects on untreated Purkinje fibers, potassium canrenoate had a prominent restorative influence on fibers treated with ouabain. Figure 7 shows action potentials from a fiber which was moderately poisoned with ouabain, as evidenced by diminution in the resting potential, amplitude, overshoot, and $V_{\text{max}}$ of the action potential (compare B with A). Potassium canrenoate produced a prompt (within 2 minutes) restoration of these parameters toward control. In this fiber and in general, a full return to the control state was not accomplished.

Even in fibers severely intoxicated with ouabain, it was possible to produce salutary changes with...
potassium canrenoate. Action potentials from a ouabain-treated fiber which became severely depolarized and inexcitable are depicted in Figure 8. A–D show progressive diminution in the resting potential, overshoot, and $V_{\text{max}}$ as well as enhancement of phase-4 depolarization until the fiber became inexcitable (D). A few minutes after potassium canrenoate was administered by bolus injection into the perfusion chamber, there was a resumption of excitability and an augmentation of resting potential, $V_{\text{max}}$, and overshoot as well as a decrease in phase-4 depolarization (E–G).

The remedial changes in resting and action potentials produced by potassium canrenoate resulted in improvement of ouabain-induced conduction block. An example of this improvement is illustrated in Figure 9. A Wenckebach type of conduction block occurred somewhere in the fibers between the site of microelectrode impalement (close to the stimulating electrodes) and the site of the bipolar electrogram (at the distal end of the fiber) (A–C). Note the successive decreases in $dV/dt$ and amplitude of the action potential during the Wenckebach cycle. After administration of potassium canrenoate, the augmented action potential was accompanied by resumption of 1:1 conduction (D and E) and shorter conduction times.

It was also possible to terminate ouabain-induced repetitive firing with potassium canrenoate. The abolition of a spontaneous rapid rhythm in a ouabain-poisoned fiber is shown in Figure 10. In this case, termination of the rapid rhythm was not associated with obvious augmentation of the resting potential or action potential of the impaled fiber. Note the positive afterpotentials, one of which appears to attain threshold (the last beat).

In six of eight ouabain-poisoned fibers, improvement occurred after administration of potassium canrenoate. Twice equimolar quantities of KC administered by bolus injection into the chamber did not affect ouabain-poisoned fibers in six experiments. In two experiments, a concentration of $10^{-6}$M ouabain was employed for more rapid intoxication and potassium canrenoate was added to the severely intoxicated fibers. No beneficial effect was observed. In three experiments, sodium canrenoate was employed in a manner similar to potassium canrenoate, and it produced comparable effects.
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The duration of the restorative effects of potassium canrenoate injected as a bolus into the tissue chamber was relatively brief (5-20 minutes). However, the ouabain infusion was continued throughout, but the potassium canrenoate was washed out of the chamber with a half time of 3-5 minutes. It was possible to repeatedly restore the same fiber with successive administrations of canrenoate, thereby prolonging the beneficial effects.

In four experiments, fibers damaged by stretch or hypoxia were exposed to canrenoate. The drug produced no effect. Records from one such experiment are shown in Figure 11.

Discussion

In intact dogs a dose of potassium or sodium canrenoate of 100-200 mg (0.25-0.5 mM) administered as a bolus intravenous injection is effective in abolishing arrhythmias induced by ouabain and digoxin within 1-2 minutes (1-4). Equimolar doses of KCl are ineffective. Assuming a dilution curve resulting from the initial transit through the pulmonary circulation and a further dilution of at least one-third resulting from diffusion from the coronary vessels into the cardiac extracellular space, it is unlikely that the peak concentration in the vicinity of the cardiac cells much exceeds $10^{-5}$M. Therefore, the concentrations employed in vivo in the present experiments (up to $5 \times 10^{-5}$M) probably encompassed the effective antiarrhythmic concentrations in vivo.

In these concentrations, canrenoate is almost devoid of electrophysiological effects on untreated Purkinje fibers. It cannot be categorized readily with other antiarrhythmic agents. It does not show the characteristic depressive features of quinidine-like or class I agents (5-7), i.e., suppression of membrane responsiveness, automaticity, and excitability. Cardiac beta-blocking properties are not evident in intact dogs, and there are no negative inotropic or chronotropic effects (2, 3).

Canrenoate cannot be classified with antiarrhythmic agents which prolong the action potential (8), nor does it fit in the recently proposed category of antiarrhythmic agents exemplified by diphenylhydantoin and lidocaine (9-12). In the concentration ranges in which class I properties are not obvious, these agents are said to be effective by enhancing conduction, particularly in Purkinje fibers depressed by various causes or in fibers excited during repolarization. This categorization has not been universally accepted (13). It has been suggested that an inappropriately low concentration of potassium in the perfusate (2.7 mM) might minimize depression of membrane responsiveness. The concentration of potassium employed in our experiments was 4.0 mM. In any case, we were unable to demonstrate any improvement of conduction by potassium canrenoate in fibers depressed...
from causes other than ouabain. Unlike diphenylhydantooin, canrenoate did not suppress the slowly rising, diminutive action potentials generated by fibers activated early during repolarization (9). It did not change the relationship between refractoriness and repolarization (Fig. 7).

The contrast between the electrophysiological impotence of canrenoate in untreated Purkinje fibers and its efficacy in ouabain-treated fibers suggests that potassium canrenoate might be a specific antagonist to the electrophysiological effects of digitalis compounds. Potassium or sodium canrenoate does not affect arrhythmias induced by catecholamines or acute coronary ligation (3). Possibly there is competition between canrenoate and digitalis for certain active sites. If this supposition is so, canrenoate is probably a relatively weak competitor, since the effective extracellular molar concentration of potassium canrenoate appeared to be greater than that of ouabain by a factor of about 1000. However, the conditions of the experiments did not allow a precise calculation of the relative concentrations at the active sites. The canrenoate was injected as a bolus into a continuously circulated bath, and its rate of diffusion to the active sites was unknown. Moreover, although the concentration of ouabain in the perfusate was known, it had been taken up by the tissue for some time before the addition of the canrenoate so that its concentration at that time at the active sites was not known. Canrenoate does not antagonize the inotropic effects of ouabain in intact animals (2, 3). Potassium ions and diphenylhydantooin also have been observed to antagonize the electrophysiological but not the inotropic effects of ouabain (13-16). This dissociation has been offered as evidence that there are different mechanisms for the electrophysiological effects and the inotropic effects of digitalis. However, conclusions relevant to mechanisms are weakened by the fact that these latter agents have antiarrhythmic electrophysiological properties independent of digitalis. Therefore, the mechanisms by which the electrophysiological effects are antagonized might be different from those by which they are produced, i.e., the antagonism might be phenomenologic rather than mechanistic. Canrenoate, because of its greater electrophysiological specificity, provides stronger evidence that mechanisms for inotropic and electrophysiological effects of digitalis are separate.

It has been postulated that the electrophysiological effects of digitalis result from the alteration of resting membrane transport of sodium and potassium (17, 18). The early effects would be explicable by changes in the concentration of potassium ion just outside the membrane, resulting in an altered electrochemical gradient and permeability of potassium. Later effects would be attributed to altered intracellular concentrations of potassium and sodium. If canrenoate is a specific antagonist of the cardiac electrophysiological effects of digitalis, then it should act to restore resting membrane transport of sodium and potassium. In the concentrations employed in these experiments, 0.5-1.5 hours were required for the electrophysiological effects of ouabain to be manifest. In contrast, the antagonistic effects of canrenoate were quite rapid. Since it is implausible to suppose that the intracellular concentrations of sodium or potassium could be significantly altered within 1-2 minutes, it would be preferable to postulate that ionic concentration gradients might be significantly augmented, because restored membrane transport might rapidly change the ionic concentrations in the immediate vicinity of the membrane. This rationalization in conformance with the hypothesis leaves unexplained the temporal disparity between transport inhibition by digitalis and transport restoration by canrenoate. There are other effects of digitalis that cannot be readily attributed to inhibition of resting membrane transport of sodium and potassium. The membrane responsiveness curve is shifted to the right (19) as with local anesthetics (5). The rate of depolarization is altered before any detectable change in resting membrane potential occurs (19, 20). These various observations suggest that digitalis might have more complicated effects on the movement of ions across the cardiac cell membrane than are explicit in the preceding hypothesis.

Restoration toward normal of fibers partially depolarized by digitalis has been accomplished by other agents. Diphenylhydantooin was reported to have salutary effects on Purkinje fibers depressed by various means (9). In this report (9), it was stated that digitalis-poisoned fibers were improved by diphenylhydantooin, but supporting data were not shown. In another report (21), doubling the extracellular potassium concentration from 2.7 to 5.4 mM produced improvement in ouabain-poisoned Purkinje fibers. The concentration of ouabain employed and the time required for onset of ouabain poisoning were similar to the values in our experiments. However, the Tyrode's solution in those experiments had a potassium concentration of 2.7 mM during the period of ouabain poisoning, whereas in our experiments the concentration was...
4.0 mM. In those experiments, the improvement required at least 10 minutes of exposure to the higher potassium, ouabain-free solution, in contrast to the rapid effects occurring with canrenoate. In our experiments, the concentrations of potassium canrenoate utilized to antagonize ouabain usually elevated the potassium ion concentration in the bath by 0.1-0.5 mM and never more than 1.0 mM. Equimolar doses of potassium chloride injected as a bolus into the bath to rapidly raise the concentration of potassium ion from 4.0 to 5.0 mM did not affect ouabain-poisoned Purkinje fibers within the first 10 minutes. Sodium canrenoate had effects comparable to those of potassium canrenoate. In a recently published study (22), both lidocaine and propranolol but not sotalol benefited ouabain-poisoned Purkinje fibers by diminishing enhanced phase-4 depolarization induced by ouabain. By restoring phase-4 depolarization toward normal, these antiarrhythmic drugs reduced the rate of firing of the ouabain-poisoned fibers and improved conduction. However, the maximum resting potential did not appear to be restored toward control by these drugs in contrast to the effect observed with canrenoate.

Spironolactone, an aldosterone antagonist like canrenoate, has been reported to counter the "neuromuscular disturbances" and mortality produced by various digitalis compounds in the rat (23). Cardiac toxicity was not specifically evaluated in these studies. It was later shown that this protective effect, which required several days of pretreatment with spironolactone, also applied to the toxicity of a variety of other compounds (24). It was found that spironolactone enhances certain hepatic enzymatic reactions important in the degradation of the toxic compounds, resulting in lower blood levels of the unchanged compound (25, 26). In addition, spironolactone by antagonizing aldosterone, might produce an increase in the serum potassium level in the intact animal, which would have an antagonistic effect on digitalis toxicity. These mechanisms, operative in the intact animal, could not have operated in our experiments.

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