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Electropharmacology of Potassium Canrenoate[1] (37880)

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Administration of digitalis in toxic concentrations will induce cardiac arrhythmias (1–5). This is such a common occurrence that the availability of a therapeutic antagonist of digitalis would be quite important.

It has been reported that potassium canrenoate [soldactone, or potassium 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl) propanate], a diuretic that specifically antagonizes aldosterone, is a therapeutic antagonist of digitalis (6–9). It was also reported that this agent at a dose of 5×10^{-5} *M* returns the ouabain-induced depression of electrophysiological characteristics of the cell membrane of dog Purkinje fibers to normal. Potassium canrenoate requires less than 1 min to restore normal cardiac function when there is a ouabain-induced tachycardia. When other authors attempted to elucidate the mechanism of this reported action of potassium canrenoate, it was found that this agent failed to alter the arrhythmogenic effects of digitalis on *in vivo* and *in vitro* hearts of dogs and rabbits, respectively, as well as cat papillary muscle (10). It was also reported that potassium canrenoate did not alter or modify the interaction of ouabain with Na^+–K^+–ATPase, the presumed site of ouabain activity on membrane phenomena (11).

In the present study we attempted to resolve these differences in reported results. If it is assumed that the response of the cell membrane reflects the locus of arrhythmias and increased cardiac pumping is a primary purpose of digitaloid use, then changes in transmembrane potential and contractile characteristics would serve as significant indices of effects of ouabain and/or potassium canrenoate for subsequent analysis. In this way we investigated the direct actions and interactions of potassium canrenoate and ouabain using isolated ventricular muscle of guinea pig.

Experimental Procedures. Strips of right ventricle (mean dimension, 0.4 mm radius; 5.2 mm long; wet wt, 14.8 mg) were taken from hearts of male guinea pigs (mean weight, 280 g). These tissues, excised according to the technique of Feigen *et al.* (12), were placed in a 17-ml temperature-controlled (30 ± 0.1°) tissue chamber filled with modified Krebs–Henseleit medium in the following composition (mmoles/l): Na^+, 145.2; K^+, 5.8; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 127.8; SO_4^{2-}, 1.2; $H_2PO_4^-$, 1.0; HCO_3^-, 27.2; and glucose 11.1 with a pH of 7.4 when aerated with 95% O_2:5% CO_2. Ventricle strips were electrically driven with threshold voltage pulses at a rate of 60/min. All experiments with these tissues used the same equipment system for recording transmembrane potential and developed tension characteristics as previously reported for right ventricle strips of guinea pigs (13).

Ventricle strips were equilibrated for at least 60 min in Krebs–Henseleit medium after insertion into the tissue chamber. The medium was replaced every 10 min during

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this period, and strips were also extended 1.2× their rest length producing a diastolic tension of 900 mg; the systolic tension averaged 266 mg. Control or normal single-cell transmembrane potential and simultaneous developed tension responses from the total tissue were recorded during the 30 min following the equilibration period. Four tissues maintained in normal Krebs–Henseleit medium were used to obtain further control data, taken at 10-min intervals for 210 min, to determine whether any tissue characteristic changed during the time period of an experiment.

Changes in transmembrane potential and developed tension characteristics of four strips each were determined under the influence of the following: (a) cumulative additions every 60 min of 3×10^{-7}, 1×10^{-6}, and 3×10^{-6} *M* ouabain; (b) 150-min exposure to 10^{-4} and 10^{-3} *M* potassium canrenoate; (c) incubation for 30 min in 10^{-4} *M* potassium canrenoate prior to administration of 3×10^{-7} and 10^{-6} *M* ouabain; and (d) induction of an ouabain-induced arrhythmia, using 3×10^{-6} *M*, followed by addition of 10^{-4} and 10^{-3} *M* potassium canrenoate. Electrical stimulation of tissues was stopped after the onset of ouabain-induced arrhythmias, demonstrating the spontaneous activity of the ventricle strip; the potassium canrenoate treatment was then initiated. All solutions were made in Krebs–Henseleit medium prior to use. Only those tissue characteristics significantly different from control values are reported.

Results. Tissue controls in normal Krebs–Henseleit medium. The mean and SEM values of the transmembrane potential and developed tension obtained from four ventricle strips during the 30-min control period are in Table I. A tracing of a typical action potential and contractile response is in Fig. 1A. Comparison of the mean tissue characteristics with those obtained during the subsequent 210-min period in normal Krebs–Henseleit indicated that there was no significant change in the action potential magnitude (AP) and the plateau ($AP\text{-}D_{20}$) and duration ($AP\text{-}D_{90}$) of the action potential measured at 20% and 90% repolarization time, respectively. However, there was a mean decrease of 15 and 30% of the developed tension (DT) which occurred about 100 and 180 min, respectively, during the 210-min test period. These changes were taken into account during subsequent analysis of the DT magnitude as influenced by the experimental procedures.

Effects of ouabain in normal Krebs–

TABLE I. Changes in Tissue Characteristics in Isolated Guinea-Pig Right Ventricle Strips Treated with Ouabain and/or Potassium Canrenoate.

	AP	$AP\text{-}D_{20}$	$AP\text{-}D_{90}$	DT
Control values in normal Krebs–Henseleit[a]	103 ± 1.0 mV[b]	102 ± 2.9 msec	202 ± 4.1 msec	267 ± 21.5 mg
Ouabain in normal Krebs–Henseleit[c]				
3×10^{-7} *M*	−13[d]	−27	−15	149
1×10^{-6} *M*	−12	−52	−32	233
Potassium canrenoate[e]				
1×10^{-4} *M*	−15	− 7	10	−48
1×10^{-3} *M*	−15	5	12	−73
Ouabain after 10^{-4} *M* potassium canrenoate[f]				
3×10^{-7} *M*	− 9	−14	− 2	126
1×10^{-6} *M*	− 3	−14	−15	295

[a] Tissues = 29; penetrations = 150; all changes expressed as percent of control.

[b] Mean ± SEM.

[c] Sixty minutes at each concentration.

[e] 150-min treatment at each concentration.

[f] 30-min potassium canrenoate incubation followed by 60 min of ouabain treatment with each concentration; % change calculated from 10^{-4} *M* potassium canrenoate control.

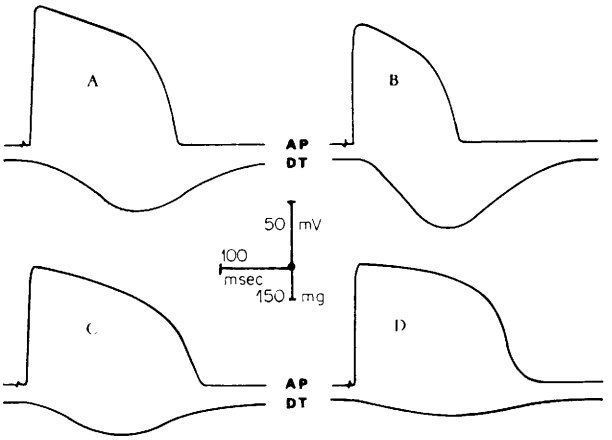

FIG. 1. Tracing of oscillographic records showing control (A), changes in action potential (AP) and developed tension (DT) under the influence of 10^{-6} *M* ouabain (B), 10^{-4} *M* (C), and 10^{-3} *M* (D) potassium canrenoate.

Henseleit medium on tissue characteristics. Ouabain in concentrations of 3×10^{-7} and 1×10^{-6} *M* (Fig. 1B) produced a marginal reduction of the AP magnitude with a dose-related shortening of the AP-D_{20} and AP-D_{90} (Table I). No statistically significant changes in the resting potential were noted during the time periods of these experiments. These ouabain concentrations also produced dose-related increases in DT magnitude (Table I). Administration of 3×10^{-6} *M* ouabain regularly induced arrhythmias with a mean intrinsic rate of 180/min within 9 ± 3.1 min (mean ± SEM). Arrhythmias invariably terminated with tissues in contracture. These tissues were unresponsive to the resumption of electrical stimulation of 60/min using as much as 10× threshold voltage.

Effects of potassium canrenoate on tissue characteristics. Administration of 10^{-4} and 10^{-3} *M* potassium canrenoate for at least 150 min induced a marginal reduction of the AP magnitude and a dose-related depression of the DT magnitude (Table I, Fig. 1C and D). There also appeared to be some variation in the repolarization configuration of the AP.

Effects of ouabain after incubation for 30 min with potassium canrenoate. Incubation of tissues with 10^{-4} *M* potassium canrenoate 30 min prior to addition of 3×10^{-7} and 1×10^{-6} *M* ouabain attenuated the depression of the transmembrane potential characteristics usually observed after administration of ouabain (Table I). However, a positive inotropy induced by ouabain was not inhibited by potassium canrenoate.

Effects of 10^{-4} and 10^{-3} M potassium canrenoate on ouabain-induced arrhythmias. Ouabain-induced arrhythmias in normal Krebs–Henseleit medium lasted for 16.4 ± 2.2 min (mean ± SEM) at which time all intrinsic activity ceased and the tissues were unresponsive to electrical stimulation. The action potential and contractile characteristics prior to cessation of all activity (Fig. 2B) were similar to those induced by increased stimulation rates. Addition of 3×10^{-6} *M* ouabain to 3 of 4 tissues pretreated for 30 min with 10^{-4} *M* potassium canrenoate did not significantly prolong the mean time to the onset of arrhythmias. The exception stopped beating within 5 min but was still arrhythmic after a brief period of electrical stimulation followed by eventual cessation of all activity. On the other hand, treatment with 10^{-3} *M* potassium canrenoate but not 10^{-4} *M* after induction of arrhythmias abolished the automaticity within 1 min, and strips once again would follow electrical stimulation at a rate of 60/min (Fig. 2C). However, the stimulus threshold had increased a minimum of twofold, and the configuration of the action

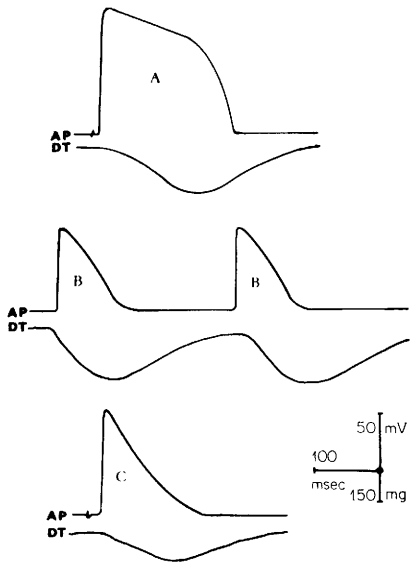

FIG. 2. Tracing of oscillographic records showing an action potential (AP) and developed tension (DT) of the control (A), under the influence of an arrhythmic concentration (3×10^{-6} *M*) ouabain (B), and within 1 min after administration of 10^{-3} *M* potassium canrenoate (C). Only sequence (B) was self-stimulated, with records taken from nonpacemaker cells. Further explanation in the text.

potential showed marked changes with an almost complete loss of the plateau phase and a severely depressed DT. Addition of 10^{-3} *M* potassium chloride prior to, during, or after administration of 3×10^{-6} *M* ouabain to induce ventricular arrhythmia did not significantly alter the responses observed in normal Krebs–Henseleit medium. Therefore, the effects of 10^{-3} *M* potassium canrenoate could not have been due to the potassium ion.

Discussion. The primary purpose of this study was to establish the electropharmacological effects of potassium canrenoate and to determine whether this agent can antagonize the toxic effects of ouabain. The toxic effects of ouabain appear to be divided into membrane and contractile phenomena. Potassium canrenoate seems to influence these events in dissimilar ways.

We found that 10^{-4} and 10^{-3} *M* potassium canrenoate decreased the action potential magnitude and increased the 90% repolarization duration in a manner similar to that observed with quinidine (14, 15). There was also a marked dose-related depression in contractile magnitude at these potassium canrenoate concentrations. These data suggest that tissue activities modified by potassium canrenoate may be produced by mechanism(s) as of yet undefined resulting in a dual action: (a) reduction of the action potential magnitude and duration through alteration of ionic permeabilities of sodium and potassium, respectively, (16, 17) and (b) a coincident dose-related depression of the contractile response.

Comparison of these results with the effects produced by additions of nontoxic concentrations of ouabain showed that the depression of the action potential magnitude and duration induced by ouabain was significantly inhibited in the presence of 10^{-4} *M* potassium canrenoate. This suggests some possibility of drug antagonism at the membrane level. Until the mechanism of potassium canrenoate-induced alteration of transmembrane potential characteristics can be related to ouabain-induced alteration of transmembrane potential characteristics and ouabain's inhibition of Na^+–K^+–ATPase, the explanation of this drug interaction on membrane events remains to be elucidated. On the other hand, it was found that the positive inotropic effects induced by $\leq 10^{-6}$ *M* ouabain were not inhibited by $\leq 10^{-4}$ *M* potassium canrenoate. This response substantiates the work of Lucchesi and Haley (10) that potassium canrenoate is not a specific antagonist as suggested by Yeh and Lazzara (8). This is in contrast to an augmented progressive negative inotropy produced by the combination of 3×10^{-6} *M* ouabain and 10^{-3} *M* potassium canrenoate. These latter results were noted only after potassium canrenoate had abolished the ouabain-induced arrhythmia and tissues were again electrically stimulated at a constant frequency.

It is well-known that the membrane potential of cardiac fibers is related to concentration gradients and passive fluxes of Na^+ and K^+ (18). Since digitalis inhibition of active transport of Na^+ and K^+ would

eventually produce a decreased concentration gradient, the net effect would result in a decreased membrane-ion gradient facilitating spontaneous depolarization as manifested by induction of ectopic foci. Ventricular arrhythmias induced by digitalis intoxication have been attributed to changes in the diastolic depolarization potential, rising velocity of phase-0 depolarization, and amplitude and duration of the His–Purkinje fibers action potentials (19–23). Although it was demonstrated that ouabain-induced changes occurred earlier in Purkinje fibers than they did in ventricular fibers (21), the latter fibers under the influence of 3×10^{-6} *M* ouabain could also become arrhythmic and show intrinsic rhythmicity with diastolic depolarization potentials (5). Therefore, a possible mechanism of ouabain-induced arrhythmias in ventricular muscle is related to initiation of phase-4 depolarization and consequent development of ectopic foci and intrinsic rhythmicity. Inhibition of ouabain-induced ectopic foci by 10^{-3} *M* potassium canrenoate may be through cessation of intrinsic rhythmicity. This antagonism by potassium canrenoate, however, occurred only with a concentration which markedly depressed contractility and did not occur with lower concentrations.

The results of this study suggest that only toxic concentrations of potassium canrenoate abolished intrinsic rhythmicity and arrhythmicity induced by ouabain. Furthermore, because of a progressive depression in contractile magnitude, it is doubtful that there is any therapeutic value of potassium canrenoate as an antiarrhythmic agent.

Summary. The action of and interaction between ouabain and potassium canrenoate were determined using changes of transmembrane potential and contractile characteristics of isolated ventricle strips of guinea pigs. Concentrations of ouabain $\geq 3 \times 10^{-6}$ *M* were always arrhythmogenic and induced intrinsic rhythmicity in normally quiescent ventricular strips. Potassium canrenoate at 10^{-4} *M* did not inhibit ouabain-induced arrhythmias or intrinsic rhythmicity. Although 10^{-3} *M* potassium canrenoate inhibited ouabain-induced intrinsic rhythmicity, there was a severe and progressive depression in the contractile magnitude. It was concluded that effective concentrations of potassium canrenoate which will inhibit ouabain-induced ventricular arrhythmias compromise the contractile function of the heart to such an extent that this drug may have little or no value in overcoming digitalis intoxication.

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