Cardiac Glycoside Effects on Rat Skeletal Muscle Potentials and Electrolytes (34426)

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The effects of cardiac glycosides on cardiac muscle electrolytes and action potentials are well known. Changes in rat skeletal muscle electrolytes induced by digitalis compounds are striking and perhaps more readily demonstrable than similar changes in cardiac muscle (1). The present study was designed to determine whether cardiac glycosides produce changes in rat skeletal muscle action potentials comparable to those of cardiac muscle and to correlate such changes with alterations in skeletal muscle electrolytes and water.

Methods. Resting membrane and stimulated intracellular action potentials were measured as previously reported, using intracellular glass microelectrodes filled with 3 *M* KCl, a Bak unity gain amplifier with cathode follower, and a Tektronix 502 oscilloscope (2) Stimuli were produced by a Grass SU4 stimulator with a SIU 4-B-RF isolation unit and delivered through wire electrodes inserted in the muscle belly. Oscilloscope traces were recorded on Polaroid film. Electrocardiograms were recorded on a Sanborn V100 machine at double sensitivity using needle electrodes in the distal limbs.

Male Sprague-Dawley rats, weighing 140–190 g, were anesthetized by intraperitoneal administration of pentobarbital for these studies. The left gastrocnemius was exposed *in situ* and covered with warm mineral oil for the electrical measurements. After exsanguination of the animal from the abdominal aorta, the right gastrocnemius was removed for measurement of muscle water, electrolytes, and inulin space. When muscle inulin space was measured, 2 ml of 20% warm inulin was injected into the peritoneal cavity 1.5 hr before the sacrifice. The analytic methods have been previously reported (2).

The animals were divided into four groups as follows:

(a) 22 control animals given 0.1 ml 0.9% NaCl subcutaneously and 5 control animals given 0.1 ml 0.9% NaCl subcutaneously at 0, 8 and 24 hr.

(b) 34 animals given 25–200 μg of ouabain

(c) 5 animals given 400–600 μg of ouabain sc.

(d) 8 animals given 100–150 μg of digitoxin sc at 0, 8, and 24 hr.

Twenty-five control animals were anesthetized and their resting membrane and action potentials were measured. The method of measuring the durations of the two phases of the intracellular action potential is illustrated in Fig. 1. The duration of A represents the period of depolarization, while the duration of B is referred to as repolarization. After the initial measurements, 0.1 ml of saline was injected subcutaneously and electrical measurements were repeated for 10–60 min. The animals were exsanguinated immediately after the last potential measurement and muscle specimens were removed for electrolyte, water, and inulin measurements. Five control animals were injected with saline at 0, 8, and 24 hr and then examined and sacrificed at 29 hr. The results in the two control groups were identical and are pooled in the tables.

The majority of group b animals were treated exactly as the control animals but were injected with ouabain after the initial electrical measurements and sacrificed at the time of maximal action potential changes. Serum and muscle analyses were not done, however, in several group b animals in which

[1] Study supported by USPHS Grants Nos. HE11980 and FR-00103.

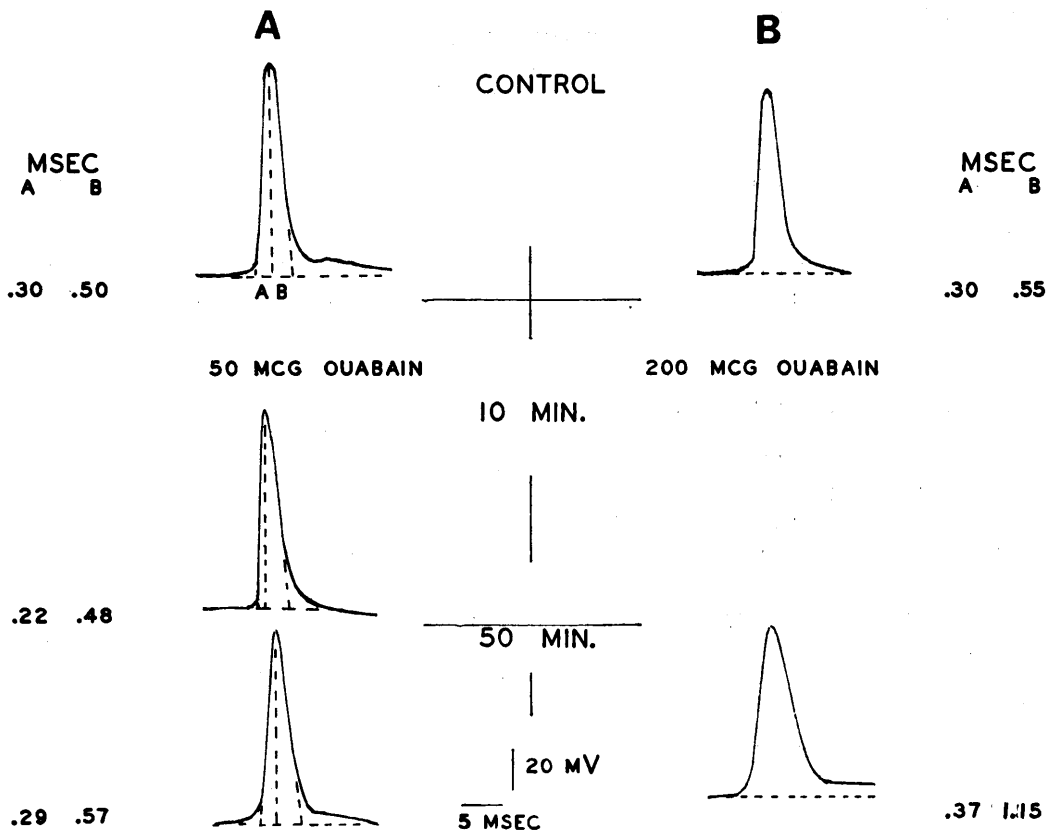

FIG. 1. Ouabain effects on rat muscle action potentials: (A) (left side) Reproductions of stimulated intracellular action potential in a rat before and 10 and 50 min after subcutaneous injection of 50 μg ouabain. (B) Reproductions of stimulated intracellular action potentials in a rat before and 50 min after injection of 200 μg of ouabain subcutaneously. A = duration of depolarization (msec); and B = duration of repolarization. Standardizations indicated are for both 1A and 1B; left gastrocnemii used; stimulus delivered by electrodes in muscle belly; resting membrane potentials were unchanged throughout.

repetitive electrical measurements were made to determine the time both of maximal action potential change and of the return of action potential to normal. Instead, muscle and serum analyses were made in an equal number of animals given the same dose of ouabain and sacrificed at times equivalent to maximal action potential changes in the previous rats.

Since the results to be reported were transient, seldom persisting for more than an hour in the group b animals, the 5 animals in group c were studied. Resting membrane potential measurements were made repeatedly in the 5 group c animals. They were sacrificed for electrolyte determination between 60–120 min after injection of ouabain. Repeat action potential measurements were also made in 4 of these animals.

Digitoxin was used in group d animals in the hope that its longer duration of activity would permit evaluation of the cumulative effects of repeated doses. Five hr after the third and final injection of digitoxin in group d animals, electrical determinations were made followed by sacrifice and collection of specimens as in the other groups.

Results. Mean muscle water and electrolyte contents are shown in Table I for the control animals, group b animals sacrificed either 3–15 min or 16–60 min after ouabain injection, group c animals sacrificed 60–120

TABLE I. Muscle Water and Electrolytes and Serum Electrolytes in Control Rats and Rats Given Cardiac Glycosides.

	Muscle					
		(meq/kg of fat-free muscle)				
	H_2O (ml/kg)	Na	K	Cl	Ca	Mg
Control rats						
mean ± SEM	772.4 ± 0.76	24.1 ± 0.54	114.2 ± 0.72	15.6 ± 0.24	3.06 ± 0.14	23.4 ± 0.27
No.	26	27	27	27	20	20
Ouabain rats						
Group b						
3–15 min	775.8 ± 0.84	24.8 ± 1.07	112.1 ± 1.03	15.8 ± 0.39	3.16 ± 0.25	23.9 ± 0.28
No.	12	12	12	12	10	10
p[a]	<.01		>.1			
16–70 min	774.6 ± 0.74	28.3 ± 0.67	111.1 ± 0.78	16.9 ± 0.24	3.22 ± 0.11	23.5 ± 0.46
No.	22	22	22	22	20	21
p	<.05	<.01	<.01	<.01		
Group c						
61–120 min	772.2 ± 0.47	30.3 ± 1.08	106.6 ± 1.33	17.3 ± 1.22	3.03 ± 0.05	23.3 ± 0.56
No.	5	5	5	5	4	4
p		<.01	<.01			
Digitoxin rats						
Group d	763.3 ± 1.73	24.9 ± 0.29	110.4 ± 1.32	13.7 ± 0.84	4.1 ± 0.85	23.8 ± 0.58
No.	8	8	8	8	8	8
p	<.01		<.02	<.05		

	(meq/liter of Serum)				
	Na	K	Cl	Ca	Mg
Control rats					
mean ± SEM	140.3 ± 0.11	4.80 ± 0.15	103.5 ± 0.49	5.17 ± 0.04	1.65 ± 0.05
No.	22	22	22	20	20
Ouabain rats					
Group b					
3–15 min	139.8 ± 0.35	5.07 ± 0.08	104.7 ± 0.55	5.17 ± 0.03	1.61 ± 0.05
No.	12	12	12	12	12
16–70 min	137.7 ± 0.61	6.56 ± 0.21	103.4 ± 0.45	5.24 ± 0.04	1.62 ± 0.03
No.	21	21	21	20	20
p	<.001	<.001			
Group c					
61–120 min	135.1 ± 2.29	8.60 ± 0.94	101.8 ± 1.24	5.16 ± 0.17	1.66 ± 0.08
No.	5	5	5	5	5
p	<.05	<.01			
Digitoxin rats					
Group d	138.3 ± 1.17	4.80 ± 0.14	102.0 ± 1.38	5.11 ± 0.11	1.68 ± 0.05
No.	8	8	8	8	8

[a] Significance of difference from control means.

min after ouabain, and group d animals sacrificed 5 hr after the last dose of digitoxin. Inulin results are not recorded as no differences were noted between the untreated and treated animals.

Muscle water varied in a biphasic manner with time after cardiac glycoside injection. For the first hour, there was a statistically significant increase in water content. The water content of muscle 61–120 min after ouabain injection was normal. Five hr after the last of three injections of digitoxin, muscle water was significantly less than in control animal muscles.

Muscle sodium was unchanged in the first 15 min after ouabain injection. Thereafter, a progressive increase in muscle sodium occurred in groups b and c. There was, however, no significant muscle sodium elevation in group d animals sacrificed 5 hr after the last of multiple digitoxin doses.

After 15 min, all treated groups had a statistically significant and equivalent decrease in muscle potassium. Muscle chloride content paralleled muscle sodium in all animal groups except those treated with digitoxin, where chloride was actually decreased significantly while sodium was unchanged.

Table I also contains serum electrolyte results. Again, there was no change in the first 15 min after ouabain. Thereafter, serum K concentration increased and serum Na decreased. These changes are statistically significant and increase with time in the acute experiments. The degree of serum electrolyte change is directly related to ouabain dose in these experiments. In the group d animals, however, there were no changes in serum electrolyte concentrations. There were no changes in concentrations of other electrolytes or osmolarity in any of the experimental groups.

Table II records the mean duration of stimulated intracellular skeletal muscle action potentials for controls and each experimental group for 7 time periods. There was a biphasic change in action potentials in relation to time after ouabain administration. This time relationship is unrelated to that of the biphasic muscle water changes. In the first 15 min there was a statistically significant reduction of duration of both depolarization and repolarization. After the first 15 min, repolarization lengthened progressively so that the total action potential became prolonged at a time when depolarization was still of normal duration. Depolarization became significantly prolonged by 50–70 min. The results in group b animals after 70 min are similar to the control values, while group c animals still demonstrated prolonged action potentials. Five hr after the third dose of digitoxin both phases of the action potential were significantly prolonged. Figure 1 is a reproduction of oscilloscope traces of action potentials in two experiments. 1A shows three action potentials recorded from an animal before, 10, and 50 min after the subcutaneous injection of 25 μg of ouabain. The duration of the action potential is reduced 10 min after ouabain injection but is normal again

TABLE II. Duration of Muscle Action Potentials.

	No.[a]	Depolarization A	Repolarization B
Controls (mean ± SEM)[b]	37/22	0.33 ± 0.01	0.71 ± 0.029
Ouabain			
Group b			
3–15 min	21/9	0.25 ± 0.015	0.59 ± 0.025
p		<.01	<.02
16–30 min	12/7	0.34 ± 0.035	0.88 ± 0.06
p			<.05
31–50 min	16/12	0.35 ± 0.029	0.85 ± 0.05
p			<.05
51–69 min	15/11	0.45 ± 0.042	1.06 ± 0.072
p		<.02	<.02
70–120 min	7/6	0.31 ± 0.014	0.89 ± 0.146
Group c			
60–120 min	4/3	1.35 (0.62–2.42)[c]	1.68 (1.14–2.42)
Digitoxin			
Group d	13/8	0.43 ± 0.03	1.02 ± 0.086
p		<.01	<.01

[a] Number of measurements/number of animals.

[b] Mean values for each rat used to calculate SEM; degrees of freedom calculated from number of animals, not from number of measurements.

[c] Range given in parentheses.

at 30 and 50 min after injection. There is no further change thereafter. 1B demonstrates prolongation of the action potential in another rat 50 min after administration of 200 μg of ouabain. Prolongation of the action potential beyond the control value was rarely demonstrable following the initial shortening in group b animals receiving 25–50 μg of ouabain. Conversely, shortening of the duration of the action potential in the first 15 min after 200 μg of ouabain was less regularly demonstrated than in the animals receiving the smaller doses. This latter difference is not significant.

Resting membrane potentials are not recorded in the tables since there was no change from the normal mean of -84 ± 0.67 mV (mean $\pm$ SEM) except in the 5 group c animals starting at 30–40 min after 400 and 600 μg of ouabain and persisting until sacrifice. The mean resting membrane potential was 69.6 mV $\pm$ 1.42 mV and simultaneous electrocardiographic changes were noted which included prolongation of the P-R and Q-T intervals, inversion of the T waves, and in one animal, bigeminy.

Action potentials were measured in 4 group c animals. There was prolongation of both phases of the action curve, noted as early as 10 min after injection. Thereafter, the action potential widened progressively, and its amplitude decreased markedly. A few measurements in the 60–90 min time period are included in Table II to give some idea of the magnitude of change.

Discussion. Group d animals who received digitoxin demonstrate that action potential prolongation is not dependent upon alteration in serum electrolyte concentrations. Serum potassium elevations after ouabain have been reported under different experimental conditions by others and have been variously attributed to hemolysis and to exchange with intracellular electrolytes (3, 4). In the present study, shortening of duration of the action potential is also independent of alterations of serum electrolytes, since it occurs before any change in electrolytes can be noted.

Shortening of the action potential is independent of demonstrable changes in muscle electrolyte and water as well. On the other hand, prolongation of repolarization coincides in time but not in degree with the decrease in muscle K content.

Working with isolated guinea pig auricles, Klaus *et al.* (3) has shown that doses of cardiac glycosides considered toxic produce two quite opposite sets of effects with the passage of time. The first and transient effect is suggested to be the equivalent of the therapeutic effect and consists of increased sodium efflux and potassium influx. The second effect, which Klaus suggests is the true toxic effect, consists of increased sodium influx and potassium efflux. The expected changes in intracellular electrolyte concentrations occur with each of these effects except that the expected increase in intracellular K is not demonstrated at the time of increased K influx. The Na efflux from normal erythrocyte ghosts has been shown in our laboratory to be similarly increased by *in vitro* exposure to ouabain in concentrations of 10^{-10} to 10^{-11} M (5). This concentration has been reported to approximate the *in vivo* therapeutic concentration (6). Conversely, ouabain inhibits *in vitro* Na efflux in concentrations of 10^{-5} M. Erythrocyte ghosts of patients receiving therapeutic doses of digitalis have increased Na efflux. The Na efflux from ghosts prepared from one patient with digitalis toxicity was reduced to normal control values (5). A similar biphasic effect on renal Na and K dependent ATPase activity, *i.e.*, stimulation by 10^{-9} M and inhibition by 10^{-4} M ouabain concentrations has been demonstrated (7).

The shortened duration of the stimulated intracellular action potential seen in the early minutes after ouabain administration represents a greater than normal rate of increase in sodium permeability during depolarization and in potassium permeability during repolarization. The mechanism for such changes is unknown. Woodbury and Hecht (8) have shown that frog cardiac muscle action potentials shorten under the influence of cardiac glycosides.

We believe that the prolongation of the action potential (as well as depression of resting membrane potential) is a toxic effect

of the cardiac glycosides. Prolonged repolarization would correlate well with the demonstrated inhibition of Na efflux and K influx with cardiac glycoside by Klaus *et al.* (3). Known inhibition of erythrocyte and renal ATPase by 10^{-5} *M* ouabain suggests that inhibition of active sodium and potassium transport may be involved in the prolongation of repolarization. On the other hand, renal Na flux does not correlate with inhibition of Na and K dependent ATPase activity (9). The prolongation of depolarization is harder to explain. It occurs without any increase in intracellular sodium so that it is not due to an altered gradient. It should not be affected by any change in active transport. We have no evidence to warrant further speculation about this mechanism.

The fact that skeletal muscle electromyography demonstrates changes sooner after ouabain than does electrocardiography is not due to the use of extracellular electrodes for the electrocardiogram, since in preliminary studies we could demonstrate equivalent changes in action potentials of skeletal muscle with extracellular electrodes. The slow recording rate and distant placement of electrodes for the electrocardiogram probably obscure early changes.

Summary. The effect of cardiac glycosides on rat skeletal muscle intracellular action potenials was studied and correlated with changes in serum and muscle electrolytes and water. In the early minutes after subcutaneous administration of ouabain, 25–200 μg, there was a significant shortening of depolarization and repolarization times. After 15-min repolarization lengthened and after 50 min depolarization also becomes prolonged. The early phase of shortening is more easily demonstrated with 25–50 μg doses, and the latter prolongation of the action potential is more marked and consistent with the larger doses.

There is also a biphasic action on muscle water content. This is apparently an increase in intracellular water as no change was seen in inulin space. The time relationship to this effect is unrelated to the time of changes in action potentials. Muscle water was increased for the first hour after injection of ouabain, returned to normal 1 to 2 hr after huge doses of ouabain, 400–600 μg, and was decreased 5 hr after the last of 3 doses of digitoxin over a 24-hr period. Muscle sodium increased significantly and progressively from 15 to 120 min after ouabain and was normal 5 hr after 3 doses of digitoxin. Muscle potassium was decreased after 15 min and became progressively lower over the next 105 min. It was also low 5 hr after 3 doses of digitoxin. Serum and muscle electrolyte and water changes are not demonstrable during the phase of shortening of the stimulated action potential. Prolongation of the action potential correlates in time only with diminution of muscle potassium content. Shortening of the action potential is thought to represent the therapeutic effect of cardiac glycosides, prolongation the toxic effect. Possible correlations with increased active sodium transport and with inhibition of Na and K dependent ATPase are discussed.

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Received July 11, 1969. P.S.E.B.M., 1970, Vol. 133.