Transmembrane Potential, Contraction and ATPase Activity of Human Heart in Relation to Ouabain*

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Summary

Effects of ouabain (10^{-12} M to 10^{-5} M) on the simultaneously recorded transmembrane action potential and contraction; and (Na^{+}-K^{+})-activated membrane ATPase of human papillary muscles were investigated. Ouabain in the concentration of 10^{-12} M did not affect the transmembrane potential, contraction and (Na^{+}-K^{+})-ATPase activity. Ouabain in the concentrations of 10^{-11} to 10^{-7} M produced a concentration dependent inhibition of (Na^{+}-K^{+})-ATPase associated with shortening of action potential duration and increase in contraction. Ouabain (10^{-7} M) produced a time dependent inhibition of the (Na^{+}-K^{+})-ATPase associated with the shortening of action potential duration and increase in contraction. Ouabain in the concentration of 10^{-5} M produced an initial marked increase in contraction which was shortly followed by a decrease in contraction and an increase in tone. The muscles developed contracture within 15 to 20 min. This concentration of ouabain inhibited the (Na^{+}-K^{+})-ATPase completely. These results suggest that there is a relation between the inhibition of the (Na^{+}-K^{+})-ATPase and shortening of action potential duration associated with an increase in contraction. Human papillary muscles seem to be very sensitive to the effect of ouabain.

Additional Indexing Words:
Adenosinetriphosphatase Human myocardium Contraction Ouabain Transmembrane potential

Cardiac glycosides have been reported to produce a shortening of the action potential duration and an increase in the force of contraction in cardiac muscle of various animal species.1-4 However, various investigators2,5,6 have disputed the relevance of this association. Hoffman and Singer7 observed no apparent correlation between the changes in the transmembrane potential and contraction. They postulated that the inotropic effects of glycosides are closely related to the contractile process itself. Dudel and Trautwein8 observed that the initial increase in the force of contraction was associated with a lengthening of APD. A further increase in the force of contraction was associated with the shortening of the action potential duration.

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Recently Mg\(^{++}\)-dependent, Na\(^+\)-K\(^+\)-activated membrane ATPase has been implicated in causing an increase in the force of contraction produced by cardiac glycosides. It has been proposed that the positive inotropic effect of cardiac glycosides is associated with the inhibition of the membrane ATPase.\(^9\),\(^10\) However, a number of investigators\(^11\)-\(^16\) have suggested that an increase in Na\(^+\)-K\(^+\)-activated ATPase activity might be correlated with the positive inotropic effect of the cardiac glycosides. This enzyme system has been reported to be stimulated by therapeutic concentrations (10\(^{-10}\) to 10\(^{-7}\) M) of cardiac glycosides.\(^11\),\(^13\),\(^15\),\(^16\) Also the stimulation produced by medium concentrations (0.5 to 5\(\times\)10\(^{-6}\) M) of cardiac glycosides has been reported to change into inhibition.\(^11\),\(^16\) The significance of the increase in the ATPase activity for contraction is not understood. Possibly it is associated with the inotropic action and the inhibitory effect at high concentration with toxic effects of cardiac glycosides. However, if so, it is hard to understand why the inotropic effects persist at high concentrations of cardiac glycosides or even medium concentration which after a prolonged incubation period inhibit the Na\(^+\)-K\(^+\)-ATPase.

Part of the difficulty in deciding about the above relationships between APD, contraction and ATPase activity arises from the failure of a single investigator to carry out the relevant measurements in the same preparation. It is, therefore, proposed to study the relationship between the positive inotropic effects of ouabain and the changes in the configuration of transmembrane potential and Na\(^+\)-K\(^+\)-stimulated membrane ATPase produced by ouabain, by studying in the human papillary muscle preparation, the transmembrane potential, contraction and ATPase activity. Human papillary muscle has been chosen because it is important to find out the effect of drug in human heart which has been changed by pre-existing heart disease and this might provide information which has therapeutic significance in the patients suffering from heart disease.

**Methods**

The experiments were carried out on papillary muscles obtained from patients aged between 15 and 50 years, undergoing corrective open heart surgery in the University of Alberta Hospital, Alberta, Canada. The methods of preparation of the muscles and recordings of contraction and transmembrane potentials were the same as described by Prasad and Callaghan.\(^17\) The muscles, after excision, were immediately transferred to a chilled Krebs-Ringer solution and removed to the laboratory for experiment. The muscle strips (length: 3 to 5 mm., width: 1 to 2 mm. and thickness: 0.5 to 0.75 mm.) were tied horizontally in a jacketed 100 ml. constant-temperature bath at 37°C. One end of the muscle was attached to a fixed holder while the other end was tied to a Grass FT-03 force displacement trans-
ducer through a movable holder in such a way as to permit isometric contraction. The resting tension was adjusted in such a way as to give maximum contraction. The Krebs-Ringer solution used had the following composition in milliequivalents per litre: Na, 138.5; K, 4.6; Ca, 4.9; Mg, 2.3; HCO₃, 21.91; PO₄, 3.48; Cl, 125; and glucose 30 mM. The solution in the bath was constantly bubbled with 95% oxygen and 5% carbon-dioxide. The muscle was stimulated supramaximally with square wave stimuli of 5 msec. duration at the rate of 60/min. by a Grass stimulator through platinum electrodes placed at one end of the muscle.

Single cell electrical activity was recorded with flexibly mounted glass micro-electrodes of tip diameter less than 0.5 μ filled with 3 M KCl having a resistance of 5 to 10 megohms. A negative capacitance electrometer was used and transmembrane potentials were displayed on a Tektronix dual beam 564A storage oscilloscope. Transmembrane potentials and contractions were simultaneously monitored on the storage oscilloscope and photographed on Polaroid film.

The muscles were equilibrated in Krebs-Ringer solution for at least 2 to 3 hours before normal transmembrane action potential and contraction were recorded. The muscles were then exposed to various concentrations of ouabain for the period of observation. Force of contraction and duration of action potential at 25% and 50% levels of repolarization were measured from photographic records.

The method of preparation of cardiac Mg⁺⁺-dependent, Na⁺-K⁺-stimulated ATPase was the same as described by Potter, Charnock and Opit.¹⁸) Papillary muscles from 2 to 3 patients were pooled together to collect at least 2 Gm. and were frozen. The tissue was blotted dry and cut into small pieces and was homogenized in 10 volumes of Tris buffer solution containing 1 mM Tris HCl, 1 mM ethylenediaminetetraacetic acid adjusted to pH 6.8. The homogenate was strained through gauze to remove connective tissue and other large tissue debris. The pH of the filtrate was adjusted to 6.8 with 0.2 M Tris solution. The homogenate was centrifuged at 1,000 × g. for 20 min. at 4°C. The supernatant was discarded and the sediment was resuspended in 15 to 20 volumes of original tissue wet weight and centrifuged at 1,000 × g. for 20 min. The supernatant was discarded and the pellet was resuspended in 1 M LiBr at low temperature (10°C) for 16 to 18 hours using 5 ml. of solution per Gm. wet weight of original tissue. The mixture was centrifuged 3 times for 20 min. at 1,000 × g., resuspending each time with Tris buffer to remove excess of lithium bromide. The final pellet was resuspended in 1 mM Tris HCl, adjusted to pH 7.8 and assayed for (Na⁺-K⁺)-activated ATPase.

Protein was determined by the method of Lowry, Rosenbrough, Farr and Randal.¹⁹) Protein determinations were performed prior to ATPase activity measurements so that equivalent amounts of protein could be utilized for study. The total volume of the enzyme assay system was 1.5 ml. and contained: 4 mM MgCl₂; 50 mM NaCl; 4 mM KCl; 4 mM Tris ATP; 50 mM histidine; 100 to 125 μg. microsomal protein, and various concentrations of ouabain. The reaction was started by the addition of the microsomal preparation and terminated by addition of 1.5 ml. of 5% trichloracetic acid. Inorganic phosphate was determined by the method described by Potter, Charnock and Opit.¹⁸) The results are expressed as micromoles of inorganic phosphate liberated/mg. protein/hr. Mg⁺⁺-dependent ATPase activity, assayed in the presence of 4 mM MgCl₂ and 4 mM Tris ATP, was subtracted from the total ATPase activity assayed in the presence of 4 mM MgCl₂, 4 mM KCl, 50 mM NaCl, and 4 mM Tris ATP. This value was the estimated
Na\(^+\)-K\(^+\) stimulated portion of the ATPase activity. Similarly the Na\(^+\)-K\(^+\) stimulated ATPase in the presence of ouabain was estimated.

**Results**

*Effect of ouabain (10\(^{-7}\) M.) on the transmembrane potential and contraction with respect to time*: The effects of ouabain in the concentration of 10\(^{-7}\) M on the transmembrane potential and contraction were recorded for a period of 75 min. A representative recording of the changes in the transmembrane action potential and contraction from a single muscle with such treatment is shown in Fig. 1. Sequential changes in the action potential duration and the force of contraction of such treated muscles are shown in Fig. 2. Within 10 min. there was a marked shortening of the duration of the action potential at all levels of repolarization and a marked increase in the force of contraction. The progressive shortening of the duration of the action potential was associated with a progressive increase in the contraction, and both of these changes were maximum within 30 to 45 min. As shown in Fig. 1, there was a decrease in the resting potential as well as in the overshoot of the action potential. After 30 to

![Fig. 1. Effect of ouabain (10\(^{-7}\) M) on the simultaneously recorded transmembrane action potentials and contractions of human papillary muscle. Control, in Krebs-Ringer solution. Arrow marks the start of the exposure of the muscle to Krebs-Ringer solution containing ouabain (10\(^{-7}\) M). The subsequent tracings are in the solution containing ouabain at intervals shown at the top of each tracing. Calibration is indicated in the figure. Note the shortening of the action potential duration associated with an increase in the force of contraction. Also note the deterioration in the transmembrane potential and contraction 75 min. after exposure of the muscle to ouabain.](image-url)
Fig. 2. Effects of ouabain (10^{-7} M) on the action potential duration (APD) at 25% and 50% levels of repolarization, contraction and Na^{+}-K^{+}-ATPase activity of papillary muscles with respect to time. Each point on the curve for APD and contraction is the mean value of 9 muscles, while that for ATPase activity is the mean value of 4 experiments. Vertical bar represents standard error. Action potential duration and Na^{+}-K^{+}-ATPase are expressed in absolute values while the contractility is expressed as percentage of control taken as 100 percent. Curve A is the Na^{+}-K^{+}-stimulated ATPase in absence, while curve B is Na^{+}-K^{+}-stimulated ATPase in the presence of 10^{-7} M ouabain. (Note the increase in the inhibition of Na^{+}-K^{+}-ATPase with respect to time). Also note that the inhibition of the Na^{+}-K^{+}-ATPase is associated with the shortening of the action potential duration and an increase in the contraction.

45 min. the force of contraction started decreasing and at the end of 60 to 75 min. the contractility approached almost to control values. At this time the transmembrane potentials were markedly affected. There was a very marked decrease in the resting potential and the overshoot of action potential associated with a marked shortening of the action potential duration. Deterioration in all these parameters was progressive. In 3 papillary muscles, 10^{-7} M ouabain produced "after contractions" after 40 min. of exposure. These after contractions were not induced by new action potentials (Fig. 3). It was also
Fig. 3. Effect of $10^{-7}$ M ouabain on the simultaneously recorded transmembrane action potential and contraction of human papillary muscle. A, control in Krebs-Ringer solution. Arrow marks the start of the exposure of the muscle to ouabain. The subsequent tracings are in ouabain at intervals shown at the top of each tracing. Calibration is indicated in the figure. Note the appearance of "after-contractions" at 40 min.

Fig. 4. Simultaneous recordings of transmembrane action potential contraction of papillary muscle. A, control in Krebs-Ringer solution. B and C, after 15 and 45 min. in ouabain. D, 120 min. after frequent washings of the muscles with normal Krebs-Ringer solution. Note that the frequent washings could not bring back the action potential and contraction to the control levels. Calibration indicated in the figure.

observed that repeated washings with normal solution even for 2 hours could not bring back the contractility and transmembrane potential to control level (Fig. 4). This was observed in 6 muscles.

Effect of various concentrations of ouabain on action potential and contraction: In human beings cardiac glycosides are effective in very low doses. Ouabain $10^{-7}$ M appeared to be toxic in the present series of experiments. It was therefore decided to use concentrations of ouabain which are either therapeutic
or more toxic. With this view in mind the effects of ouabain in concentrations of $10^{-12}$, $10^{-11}$, $10^{-10}$, $10^{-9}$, $10^{-7}$, and $10^{-5}$ M on the transmembrane potential and contraction were investigated for a period of 1 hour. Representative recordings of the changes produced by $10^{-11}$, $10^{-10}$, and $10^{-9}$ M of ouabain are shown in Figs. 5, 6, and 7 respectively. It was observed that the shortening

Fig. 5. Effects of ouabain ($10^{-11}$ M) on the simultaneously recorded transmembrane action potential and contraction of human papillary muscle. Control, in Krebs-Ringer solution. Subsequent tracings are in ouabain ($10^{-7}$ M) at intervals shown at the top of each tracing. Calibration is indicated in the figure.

Fig. 6. Effects of ouabain ($10^{-10}$ M) on the simultaneously recorded transmembrane action potential and contraction of human papillary muscle. A, control in Krebs-Ringer solution. Subsequent tracings B, C, and D are after 15, 45, and 60 min. respectively in ouabain. Calibration indicated in the figure.

Fig. 7. Effects of ouabain ($10^{-9}$ M) on the simultaneously recorded transmembrane action potential and contraction of human papillary muscles. A, control in Krebs-Ringer solution. Subsequent tracings B, C, and D are after 30, 45, and 60 min. in ouabain $10^{-9}$ M.
of the duration of the action potential associated with an increase in the force of contraction appeared within 15 min. and these changes gradually progressed to reach their maximum within 45 min. after which either there was a slight further increase in the contraction and shortening of the action potential duration (Figs. 6 and 7) or a slight decrease in the contraction and increase in the APD (Fig. 5). The shortening of the duration of action potential and increase in the force of contraction were dependent upon the concentrations of ouabain (Fig. 8). Ouabain in the concentration of $10^{-11}$ and $10^{-10}$ M did not produce any apparent change in the resting potential and overshoot of action potential duration (APD) at 25% level of repolarization, contraction and Na$^+$–K$^+$-ATPase activity of human papillary muscles. Each point on the curve for APD and contraction is the average of 9 experiments, while that for ATPase activity is mean for 5 experiments. Vertical bar represents standard error. Action potential duration and Na$^+$–K$^+$-ATPase activity are expressed in absolute values, while contraction is expressed as percentage of control taken as 100 percent. Contraction and APD shown in the figure have been measured after 45 min. in ouabain. Note the inhibition of the ATPase is associated with the corresponding shortening of the action potential duration and the increase in the contraction.
potential (Figs. 5 and 6), while in the concentration of $10^{-9}$ M produced a slight decrease in the resting potential and overshoot of action potential (Fig. 7). Ouabain in the concentration of $10^{-5}$ M produced a marked increase in the contraction associated with a marked shortening of the action potential within 2 min. and these changes persisted only for 5 to 10 min. after which deterioration in all these parameters was highly progressive until finally within 15 to 20 min. there was cardiac arrest and inexcitability of the tissue. Ouabain in the concentration of $10^{-12}$ M was ineffective in producing changes in either the contraction or the transmembrane potential.

Effect of time of incubation on the ouabain-induced inhibition of $(Na^+-K^+)$-ATPase:

Since $(Na^+-K^+)$-ATPase inhibition has been implicated in the ouabain-induced positive inotropic effects in the heart and since there is a time-dependent progressive increase in the contraction till it almost reaches its maximum, it was desirable to investigate the effect of ouabain on the $(Na^+-K^+)$-ATPase with respect to time of incubation. Four experiments were conducted in which the effects of ouabain in the concentration of $10^{-7}$ M on the $(Na^+-K^+)$-ATPase activity of the papillary muscles were investigated at intervals of 10, 20, 30 and 45 min. of incubation. Simultaneous measurements of $(Na^+-K^+)$-ATPase without ouabain were also made. The results are summarized in Fig. 2. The $(Na^+-K^+)$-ATPase activity in the absence of ouabain was linear and reached $5.66 \pm 1.0$ (S.E.) $\mu$M/mg. protein/hr. at the end of 45 min. Ouabain, with increasing time of exposure, produced progressively greater inhibition of $(Na^+-K^+)$-ATPase of the cardiac muscle.

Effect of varying concentrations of ouabain on $(Na^+-K^+)$-ATPase: Repke has reported that strophanthin in higher concentrations ($10^{-7}$ to $10^{-4}$ M) produced concentration-dependent inhibition of the $(Na^+-K^+)$-ATPase. He also demonstrated that lower concentrations ($10^{-10}$ to $10^{-8}$ M) of ouabain which are, in fact, in a therapeutic range, produced stimulation of the $(Na^+-K^+)$-ATPase. In the present series of experiments it was observed that ouabain produced an increase in the contraction in the concentration as low as $10^{-11}$ M. It was therefore decided to study the effect of various concentration ($10^{-12}$, $10^{-11}$, $10^{-10}$, $10^{-9}$, $10^{-7}$, $10^{-5}$, and $10^{-4}$ M) of ouabain on the $(Na^+-K^+)$-ATPase of the human papillary muscles. Since near maximal contractility developed after 45 min. exposure of the muscle to ouabain, the incubation period for ATPase activity was 45 min. The results are summarized in Fig. 8. Ouabain $10^{-12}$ M produced neither stimulation nor inhibition of the $(Na^+-K^+)$-ATPase. Ouabain in the concentration of $10^{-5}$ M completely inhibited the $(Na^+-K^+)$-activated ATPase. There was a concentration-dependent inhibition of the $(Na^+-K^+)$-activated ATPase.
DISCUSSION

The results with $10^{-7}$ M ouabain on the duration of action potential, contraction and (Na$^+$-K$^+$)-ATPase activity of human papillary muscles indicated that there was a time dependent relationship between the inhibition of the (Na$^+$-K$^+$)-ATPase, shortening of the action potential duration and the increase in the cardiac contraction. With the progressive inhibition of the (Na$^+$-K$^+$)-ATPase there was a concomitant progressive shortening of the action potential duration and an increase in the contraction (Fig. 2). The results also indicated that ouabain $10^{-12}$ M was not effective in producing any change in the transmembrane potential, contraction and (Na$^+$-K$^+$)-ATPase activity. Ouabain in the concentration of $10^{-12}$ to $10^{-7}$ produced a concentration-dependent inhibition of the (Na$^+$-K$^+$)-ATPase, shortening of the action potential duration and increase in the cardiac contraction. Ouabain $10^{-5}$ M completely inhibited the (Na$^+$-K$^+$)-ATPase and produced inexcitability in the cardiac muscles.

The increase in the cardiac contraction associated with shortening of the duration of action potential produced by cardiac glycosides has been reported in various animal species. The initial increase in the duration of action potential associated with an increase in the contraction produced by ouabain as reported by Dudel and Trautwein in cat was never observed in the present series of experiments in any concentrations. Onset of inotropic effects in sheep ventricle and guinea pig auricle has been reported following cardiac glycosides prior to any observable changes in the configuration of action potential. In the present series of experiments ouabain in all the concentrations which increased contraction produced a shortening of the action potential duration. Not only that but also the increase in the contraction and shortening of the action potential duration were time and concentration dependent. Lowest concentration ($10^{-12}$ M) of ouabain used in this series produced changes neither in the transmembrane potential nor in the contraction. In this context it is worthwhile mentioning that low potassium, low sodium, and calcium which are known to inhibit membrane ATPase, have been reported to produce a shortening of the duration of action potential and increase in the force of contraction in cardiac muscle of man and guinea pig.

In toxic concentrations the cardiac glycosides have been reported to produce disturbances in the configuration of transmembrane potential. The initial change was a decrease in the total duration of action potential, made of a diminished plateau and increase rate of repolarization. This was followed by a decrease in the magnitude of resting potential. This was followed by a
further decrease in the rate of rise and the amplitude of action potential. The deterioration in all these parameters was progressive until finally there was cardiac arrest and inexcitability of the tissue. In the present series of experiments, no change in resting potential or overshoot of action potential except shortening of the APD was observed during the positive inotropic effects with lower concentrations of ouabain. In higher concentrations of ouabain, however, the changes observed by Kassebaum9) in the transmembrane potential were also observed in the present series of experiments. Ouabain in the concentrations of $10^{-7}$ and $10^{-5}$ M produced a decrease in the contraction following an initial increase in the present series of experiments. Results similar to this have been reported by Lee et al.26) in cat papillary muscles in the toxic concentration of $1.37 \times 10^{-6}$ M of ouabain after 120 min. Human papillary muscles seem to be very sensitive to the effects of ouabain because $10^{-7}$ M ouabain produced a decrease in the contraction after 40 to 45 min. and $10^{-5}$ M ouabain produced a cessation of contraction within 15 to 20 min. “After-contractions” not induced by a new depolarization similar to that observed in human papillary muscle in the present experiment, have also been observed in guinea pig heart in toxic concentration ($2 \times 8 \times 10^{-5}$ M) of ouabain.27)

The (Na+-K+)-ATPase of human heart seems to be very sensitive to ouabain. As low as $10^{-11}$ M produced an appreciable inhibition of the (Na+-K+)-ATPase and the concentration of $10^{-5}$ M completely inhibited the (Na+-K+)-ATPase. Strophanthin K in the concentrations of as high as $10^{-8}$ M16) and $10^{-10}$ M13) in guinea pig heart have been reported to stimulate (Na+-K+)-ATPase. These investigators observed that strophanthin K $10^{-5}$ M produced approximately 75% inhibition only. Ouabain in none of the concentrations stimulated the (Na+-K)-activated ATPase as opposed to observed by various other workers.11)-16) The concentration $10^{-12}$ M of ouabain which did not produce any change in the (Na+-K+)-ATPase also did not produce any change in the transmembrane potential and contraction. The concentrations of ouabain which produced an inhibition of the (Na+-K+)-ATPase also produced an increase in the contraction. These results are in agreement with those observed by Besch et al.16) and Akera et al.9) in dog heart in vivo.

Assuming the genesis of repolarization as a result of efflux of potassium during activity28) the shortening of the duration of action potential produced by ouabain might be due to an increased efflux of potassium because of an inhibition of the membrane (Na+-K+)-ATPase. An increased loss of potassium associated with the shortening of the action potential duration during positive inotropic effect of ouabain has been reported by Müller.21) Trautwein and Dudel,20) MacLeod and Prasad30) and Prasad and Callaghan17) reported a shortening of the action potential in cardiac muscle of various
animals including man in the presence of metabolic inhibitors. They interpreted their results as an increased efflux of K+ during activity. It has been reported by Prasad and MacLeod21) and Prasad20) that for the maintenance of normal action potential duration it is not only the energy but the normal ATPase activity which is also essential. Thus low potassium, low sodium, high calcium which are also known to inhibit membrane ATPase13),16),22) have been reported to produce a shortening of the action potential duration and an increase in the force of contraction.3),21),23),25) Also potassium and rubidium which are known to stimulate membrane ATPase22) have been reported to produce a lengthening of the action potential duration and decrease the force of contraction in the human cardiac muscles equilibrated in KCl-free solutions24). It thus seems that an increased efflux of K+ caused by procedures which inhibit either energy production or membrane ATPase is usually associated with a shortening of the action potential duration.

The increase in the force of contraction produced by ouabain might be due to an increased influx of Ca++ and an increased availability of ATP, not utilized for the active transport of Na+ and K+, for cardiac contraction as a result of inhibition of membrane ATPase. Ouabain has been shown to increase the rate of Ca++ uptake by cardiac tissue.31)33) Also an increased efflux of K has been reported to be associated with an increased access of Ca++ to the contractile site.34) Baker et al.35) have demonstrated that an increase in the internal Na+ in squid axons increased the Ca++ influx. It seems that the inhibition of (Na+-K+)-ATPase is somehow related to the increased efflux of K+ and an increased influx of Ca++. Inhibition of (Na+-K+)-ATPase might increase the intracellular concentration of Ca++ by interfering with the uptake of Ca++ by sarcoplasmic reticulum.36) If this is the case the ouabain would increase the duration of contractile process, but cardiac glycosides have been reported to produce an increase in the rate at which tension or force develops.37) Thus, this mechanism of action of cardiac glycoside might be in question. Cardiac glycosides might increase the amount of Ca++ released from the reticulum by inhibiting the membrane ATPase. As a matter of fact, Chipperfield and Nayler38) have demonstrated an increase in the Ca++ release from reticulum by electrical stimulation. Inhibition of (Na+-K+)-ATPase would increase the intracellular Na+ concentration which in turn might release Ca++ from the membrane. Sodium ion has been demonstrated to release Ca++ from brain microsomes.39) The release of Ca++ from the cardiac sarcoplasmic reticulum or other particulates by sodium has not yet been demonstrated.

The present study demonstrated that ouabain inhibited the (Na+-K+)-ATPase isolated from human heart and this effect was associated with a shortening of the action potential duration concomitant with an increase in the force
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