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MECHANISMS OF REDUCED DIGITALIS TOLERANCE WITH ADVANCING AGE IN THE GUINEA PIG

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Abstract—In the present study, arrhythmogenic toxicity of cardiac glycoside ouabain was investigated in guinea pigs after intravenous infusion (5 μg/kg/min). Guinea pigs of 18–24 months of age required significantly ($P < 0.05$) lower doses of ouabain than 3-month-old animals (72 ± 3 vs 100 ± 3 μg/kg) for the initiation of cardiac arrhythmias. Investigation of Na^+–Ca^{2+} exchange in the isolated sarcolemmal vesicles revealed a marked reduction in the Na^+-dependent Ca^{2+} uptake. Kinetic analysis of these data has demonstrated a 70% reduction in V_{max} and reduced affinity for Ca^{2+} in vesicles from 18-month-old as compared to 3-month-old guinea pigs. The rate of Na^+-dependent Ca^{2+} efflux was also markedly lower in the vesicles of older animals, and the vesicles retained more Ca^{2+} after 3 min of Na^+-dependent Ca^{2+} extrusion than did those from 3-month-old animals. The results suggest that the sensitivity to cardiac glycocide increases with age and may be associated with altered sarcolemmal Na^+–Ca^{2+} exchange activity.

Despite the advent of new inotropes and vasodilators, administration of digitalis still remains the mainstay of treatment of congestive cardiac failure. Treatment with digitalis is often complicated by the development of cardiac arrhythmias due to its narrow therapeutic index [1]. Manifestation of cardiac toxicity is increased further in elderly patients, resulting in a narrow margin of safety for digitalis [2, 3]. The increased sensitivity of the myocardium of the older animal to digitalis has also been reported in experimental animals of different species [4–6]. Variation in the metabolism of digitalis within the different age groups did not seem to be a major contributor to the difference in digitalis tolerance [7, 8]. Furthermore, the manifestation of arrhythmias in the elderly patients does not correlate directly with the plasma digoxin levels [9]. These reports suggest that the increased sensitivity to digitalis is due most likely to the changes in the myocardium with age.

Many reports, including reports from our laboratory, have suggested that the digitalis-induced arrhythmias involve a state of "calcium overload" [10–14] resulting from enzyme (Na^+-K^+-ATPase) inhibition [15, 16] and a subsequent increase in Ca^{2+} influx via Na^+–Ca^{2+} exchange [17, 18]. Recently, it has been suggested that the reduction of Ca^{2+} efflux due to an increased $[Na^+]_i$ may also contribute to the elevation of cytoplasmic calcium in digitalis toxicity [19]. In either way, the Na^+–Ca^{2+} exchange is an important intermediate step between enzyme inhibition and elevation of cytoplasmic calcium, which may rise to a level of "calcium overload" resulting in digitalis-induced arrhythmias. With these observations, it is entirely logical to hypothesize that the alterations in Na^+-dependent Ca^{2+} exchange (which ultimately increases cytoplasmic calcium) of the older animal heart may be responsible for the increased sensitivity to digitalis-induced arrhythmias.

Our earlier studies in guinea pig hearts have demonstrated that there is an age-dependent progressive reduction in the activity of enzyme Na^+–K^+-ATPase and over 50% reduction in binding sites in aging from 6 weeks to 24 months [5]. The objective of the present study was to investigate digitalis tolerance in the aging guinea pigs by using a whole animal model and to study the state of the Na^+–Ca^{2+} exchanger in the sarcolemmal vesicles isolated from these older animal hearts.

METHODS

Male guinea pigs (*Cavia porcellus*), age ranging from 3 to 24 months, were used in these experiments. The breeders were purchased from Charles River, Quebec, Canada, and bred in a controlled environment at the animal centre of the campus. These guinea pigs attain sexual maturity at 3 months and reach senescence (50% mortality) at 24 months.

Effects of ouabain in anesthetized animals

Guinea pigs, 18–24 months old, weighing 900–1000 g were investigated for arrhythmogenic toxicity of ouabain and compared with guinea pigs that were 3 months old (250–300 g).

Instrumentation. Animal preparation and measurements of basal parameters were carried out by methods described earlier [11]. Briefly, animals were anesthetized with α-chloralose (60 mg/g) and urethane (500 mg/kg) intraperitoneally. Tracheostomy was performed, and the animals were ventilated mechanically with room air, using a Harvard respirator (model 680). The stroke volume of the pump was adjusted according to the body weight. The chest was cut open with minimal bleeding, and a

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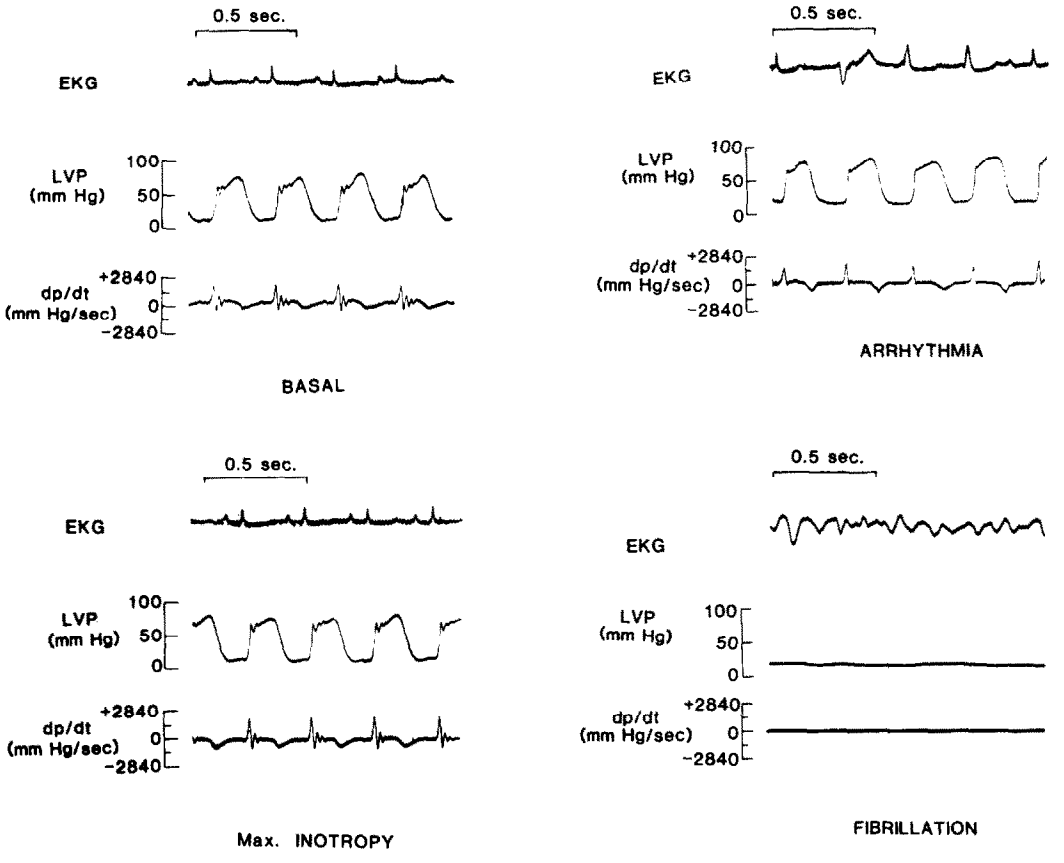

Fig. 1. Simultaneous recording of EKG, LVP and its dp/dt in 18-month-old guinea pigs. Recordings illustrate the basal parameters (top left) and changes during ouabain infusion (5 μg/kg/min): inotropy (bottom left), arrhythmias (top right), and fibrillation (bottom right).

fluid-filled (heparinized saline) cannula was inserted into the left ventricle through the apex and placed *in situ* without any interference to the left ventricular function. Left ventricular pressure and the rate of its rise (dp/dt) were recorded using a Statham P23Gb transducer and a Hewlett–Packard recorder (model 1308A). The external jugular vein was cannulated for the infusion of drugs during the experiment. Lead II EKG was monitored by the Hewlett–Packard recorder throughout the experiment.

Infusion of ouabain. After all the surgical procedures were completed, 15–20 min was allowed for stabilization before any physiological recordings were taken. Then ouabain was infused at a rate of 5 μg/kg/min in saline (26 μL/min) via the external jugular vein by using a Harvard infusion pump (model 600-000) until fatal cardiac arrhythmias appeared. The appearance of fatal cardiac arrhythmias were characterized by 6–8 successive ventricular ectopics leading to sustained ventricular tachycardia and/or ventricular fibrillation.

Na^+–Ca^{2+} *exchange in sarcolemmal vesicles*

Na^+–Ca^{2+} exchange was studied in the sarcolemmal vesicles isolated from guinea pig ventricles of three different age groups (3 months, 6 months, and 18 months).

Preparation of sarcolemmal vesicles. Sarcolemmal vesicles were prepared as described by us earlier [20]. Briefly, guinea pig heart left ventricles from three to four animals were homogenized in 0.6 M sucrose, 10 mM imidazole/HCl, pH 7.0 (4 vol./g tissue) using a Polytron PT-20 (4 × 15 sec; setting 5) and centrifuged at 10,000 *g* for 20 min. The supernatant fraction was diluted (2-fold) with 160 mM NaCl/20 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), pH 7.4, and centrifuged at 96,000 *g* for 60 min. The pellet was resuspended in NaCl/MOPS, layered over 15 mL of 30% sucrose solution containing 0.3 M NaCl, 50 mM sodium pyrophosphate, and 0.1 M Tris-HCl (pH 8.3) and centrifuged at 95,000 *g* for 90 min. The white band at the sample sucrose interface was recovered, diluted with 3 vol. of NaCl/MOPS, and centrifuged at 100,000 *g* for 30 min. The pellet was resuspended in NaCl/MOPS to a final protein concentration of approximately 1 mg/mL. The Na^+-loaded vesicles were prepared by the above method, and the K^+-loaded vesicles were prepared by substituting 160 mM KCl/MOPS for 160 mM NaCl/MOPS. The protein concentrations were determined by the method of Lowry *et al.* [21].

Na^+-*dependent* $^{45}Ca^{2+}$ *uptake.* Vesicles were preloaded with Na^+ by incubating them in 160 mM NaCl/20 mM MOPS (pH 7.4) for 60 min at 37°. To initiate Na^+–Ca^{2+} exchange, aliquots of 20 μL

Table 1. Basal heart rates and left ventricular parameters in adult and aged guinea pigs

Age of animals (months)	Heart rate (beats/min)	Left ventricular pressure (mm Hg)	+dp/dt (mm Hg/sec)	−dp/dt (mm Hg/sec)
3	260 ± 11	69 ± 5.6	2777 ± 140	1420 ± 100
18–24	213 ± 6*	66 ± 4.2	1917 ± 178*	944 ± 81*

Values are means ± SE of six to eight experiments.
* Significantly different from 3-month values ($P < 0.05$).

(equivalent to 20 μg protein) of Na^+-loaded vesicles were added to a series of tubes containing an incubation mixture (160 mM KCl/20 mM MOPS, pH 7.4, at 37°) plus 50 μM $^{45}CaCl_2$ (100 cpm/pmol) in a volume of 500 μL. The exchange was terminated by adding the "termination solution" (2 mL ice-cold 160 mM KCl/20 mM MOPS/1 mM $LaCl_3$, pH 7.4) at desired times followed by filtration through Millipore filters (0.45 μm) under vacuum. Tubes and filters were rinsed four times with 2 mL of "termination solution." The filters were placed into scintillation vials, 10 mL of a scintillation fluid was added, and the radioactivity was counted in a Beckman LS 8100 liquid scintillation counter [20]. In all experiments, non-specific $^{45}Ca^{2+}$ uptake was determined in the vesicles that were loaded with potassium (160 mM KCl/20 mM MOPS, pH 7.4) instead of sodium.

In another set of experiments, sarcolemmal vesicles isolated from 3- and 18-month-old guinea pigs were first loaded with sodium and then incubated in the incubation mixture (160 mM KCl/20 mM MOPS, pH 7.4, at 37°) with various concentrations of $^{45}Ca^{2+}$, ranging from 20 to 80 μM. The Na^+-dependent Ca^{2+} uptake was terminated after 5 sec of incubation. The filtration and counting of $^{45}Ca^{2+}$ on the filter were carried out as described above.

Na^+-dependent $^{45}Ca^{2+}$ efflux. Na^+-loaded vesicles were allowed to accumulate $^{45}Ca^{2+}$ for 1 min in 160 mM KCl/20 mM MOPS (pH 7.4) plus 50 μM $^{45}Ca^{2+}$ in a volume of 500 μL. Calcium efflux was then initiated by increasing the Na^+ concentration of the medium to 90 mM. The exchange was terminated at desired times and filtered through the Millipore filters, as in the $^{45}Ca^{2+}$ uptake study, and the quantity of $^{45}Ca^{2+}$ was counted by liquid scintillation.

Reagents

Analytical grade chemicals dissolved in deionized glass-distilled water were used throughout. Ouabain-octahydrate was purchased from the Sigma Chemical Co., St. Louis, MO (U.S.A.).

Statistical analysis

Data are expressed as means ± SE. Student's *t*-test or analysis of variance was used wherever appropriate for statistical analysis, taking $P < 0.05$ as the level of significance.

RESULTS

Basal cardiovascular parameters

Basal electrocardiographic and haemodynamic parameters were recorded after 15–20 min of stabilization. A typical basal recording from an 18-month-old guinea pig is shown in Fig. 1 (top left), and the analysis of these recordings is given in Table 1. The basal heart rate of an 18- to 24-month-old guinea pig was 18% lower than that of a 3-month-old, and there was no change observed in the PR interval between the two age groups [11]. The bradycardia in older animals was demonstrated in both anesthetized (213 ± 6 vs 260 ± 11 beats/min) and conscious (209 ± 15 vs 257 ± 9 beats/min) guinea pigs. The systolic left ventricular pressure (LVP) of the older animals was similar to the LVP of the younger guinea pigs. However, the +dp/dt and −dp/dt, which measure the left venticular contractility and relaxation, were reduced substantially in the older guinea pigs.

Arrhythmogenic toxicity of ouabain

In the control (3-month-old) animals, infusion of ouabain at a rate of 5 μg/kg/min produced an initial inotropic response (manifested as increase in dp/dt and elevation of LVP) in 7–9 min of infusion, and a ouabain dose of 43 ± 6 μg/kg produced a maximum increase in +dp/dt of 43.33% (Table 2). On the other hand, the older guinea pigs (18–24 months) developed the maximum inotropic response of only 25% and required 28 ± 2 μg/kg of ouabain. The continuous infusion of ouabain to the control animals initiated ventricular ectopics in 20–22 min. A dose of 100 ± 3 μg ouabain/kg was necessary to initiate arrhythmias, and 140 ± 4 μg/kg was lethal to this group of animals (Table 2). The older guinea pigs, on the other hand, developed arrhythmias within 15 min of ouabain infusion. A dose of ouabain of only 72 ± 3 μg/kg initiated ventricular ectopy, which quickly progressed to ventricular tachycardia and/or ventricular fibrillation. A dose of 93 ± 5 μg ouabain/kg was lethal to these older guinea pigs (Table 2). Changes in heart rates and PR intervals with the infusion of ouabain, however, were not significantly different between the two age groups.

Na^+–Ca^{2+} *exchange*

Na^+-dependent $^{45}Ca^{2+}$ uptake. The uptake of $^{45}Ca^{2+}$ was studied using Na^+-loaded vesicles. $^{45}Ca^{2+}$ uptake in K^+-loaded vesicles was taken as non-specific and thus subtracted from the respective values of Na^+-loaded vesicles to obtain net Na^+-dependent calcium uptake. The time–courses of Na^+-dependent $^{45}Ca^{2+}$ uptake in the presence of 50 μM $^{45}Ca^{2+}$ for vesicles of three different age groups are shown in Fig. 2. In all three groups of

Table 2. Effects of ouabain infusion (5 μg/kg/min) in young adult and aged guinea pigs

Age of animals (months)	Inotropic response: Dose of ouabain (μg/kg)	Inotropic response: % Maximum increase in +dp/dt	Toxic response: Arrhythmogenic dose of ouabain (μg/kg)	Toxic response: Lethal dose of ouabain (μg/kg)
3	43 ± 6	43.33 ± 6	100 ± 3	140 ± 4
18–24	28 ± 2*	25.00 ± 4*	72 ± 3*	93 ± 5*

Values are means ± SE of six to eight experiments.
* Significantly different from 3-month values ($P < 0.05$).

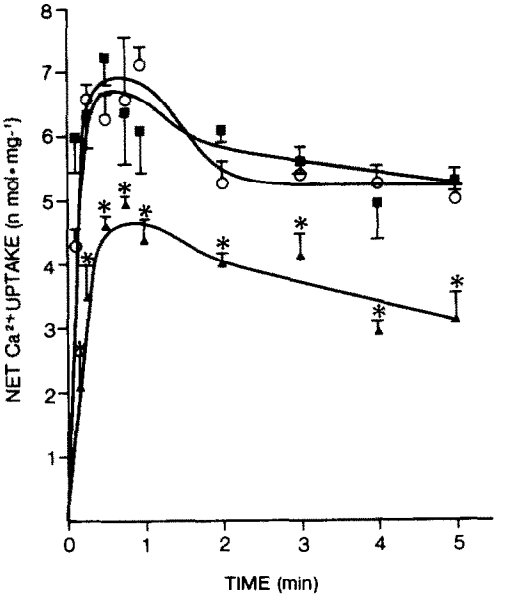

Fig. 2. Time-course of Na^+-dependent Ca^{2+} uptake in sarcolemmal vesicles of 3- (○—○), 6- (■—■) and 18- (▲—▲) month-old guinea pigs. The $[Ca^{2+}]_o$ was 50 μM. Each value is the mean ± SE of five experiments carried out in duplicate in five independent membrane preparations. Key: (*) significantly different from the appropriate control (3-month) values ($P < 0.05$).

vesicles, there was an initial rapid uptake of $^{45}Ca^{2+}$ followed by a plateau phase. However, substantial differences were seen in the amount of $^{45}Ca^{2+}$ taken up both in the initial period and in the plateau phase. The maximum uptake of $^{45}Ca^{2+}$ in vesicles of the control hearts (3 months) was seen within 15–30 sec, and the $^{45}Ca^{2+}$ content of the vesicles at this time was 6.4 ± 0.34 nmol/mg protein. In the following 1–1.5 min, calcium content of the vesicles was reduced slowly and reached a steady state within 2 min. On the other hand, the vesicles of the older animal hearts (18 months) accumulated calcium at a relatively slower rate with substantially lower maximum accumulated calcium. The calcium content of these vesicles at 45 sec was 4.63 ± 0.05 nmol/mg protein which then was reduced slowly to 3.13 ± 0.4 nmol/mg protein at 5 min. The Na^+-dependent Ca^{2+} uptake in the vesicles of 6-month-old guinea pigs did not show any significant difference in the amount of

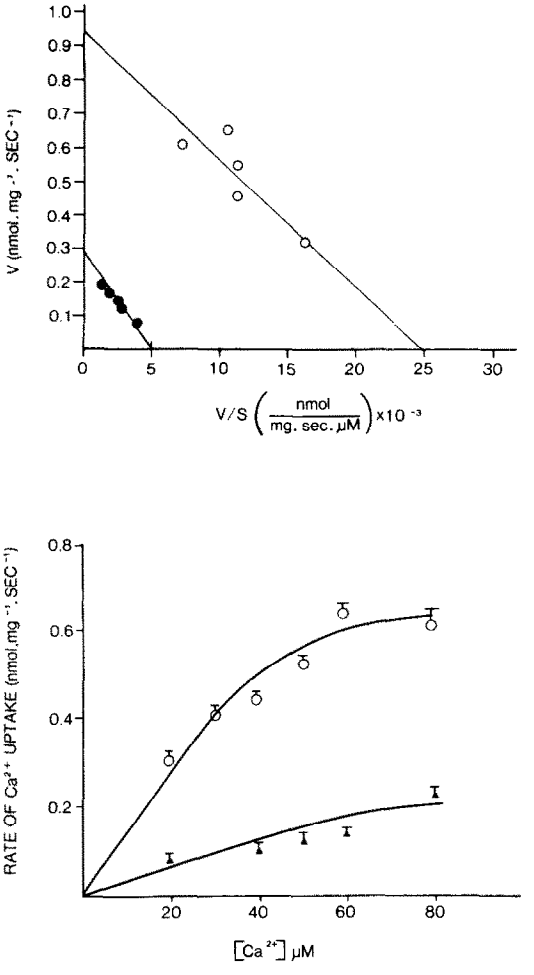

Fig. 3. Top panel: Eadie–Hofstee plot of calcium uptake. V: rate of calcium uptake measured in 5 sec of incubation and calculated per second. V/S: rate of uptake/concentration of $^{45}Ca^{2+}$. The data represent typical experiments performed in duplicate. The line was determined by linear regression analysis. The Michaelis–Menten constant (K_m) was calculated from the slope, and the maximum initial velocity (V_{max}) was determined from the intercept of the line with the ordinate. Experiments were repeated five times with independent membrane preparations from (○—○) 3- and (●—●) 18-month-old guinea pigs. Bottom panel: Rate of Na^+-dependent $^{45}Ca^{2+}$ uptake as a function of $[Ca^{2+}]_o$ ranging from 20 to 80 μM. Each value is the mean ± SE of five experiments carried out in duplicates with independent membrane preparations from (○—○) 3- and (▲—▲) 18-month-old guinea pigs.

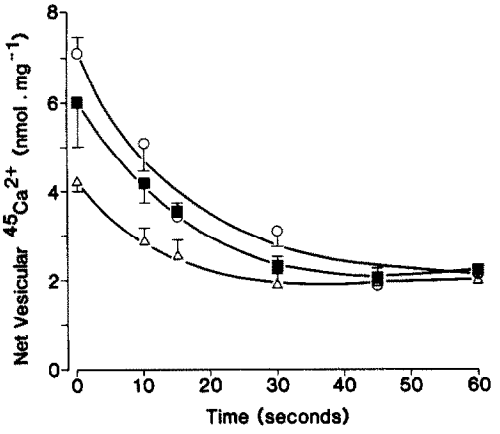

Fig. 4. Time–course of Na^+-dependent Ca^{2+} efflux in sarcolemmal vesicles of 3- (○—○), 6- (■—■), and 18- (△—△) month-old guinea pigs. The efflux was initiated after 1 min of Na^+-dependent $^{45}Ca^{2+}$ influx in 50 μM $^{45}Ca^{2+}$ (as in Fig. 2) and transferring the vesicles into 90 mM Na^+ medium. Each value is the mean ± SE of five experiments, carried out in duplicate in five membrane preparations. The initial rates in older animals determined during 5 sec were significantly ($P < 0.05$) different from those in 3- to 6-month-olds.

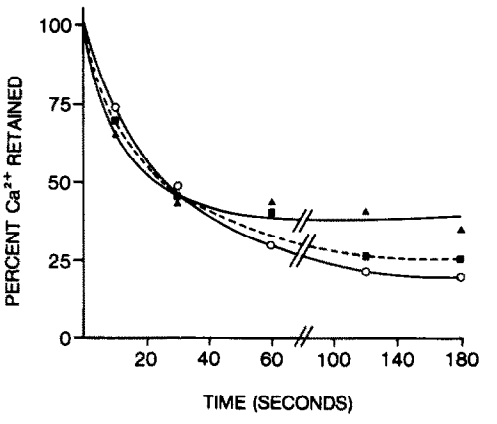

Fig. 5. Time–course of percent $^{45}Ca^{2+}$ remaining after incubation of vesicles in high Na^+ (90 mM) medium. The percent $^{45}Ca^{2+}$ retention indicates the amount of $^{45}Ca^{2+}$ at a particular time in proportion to its initial content at zero time. The data represent typical experiments performed in duplicate. Experiments were repeated five times with independent membrane preparations. Key: vesicles from 3- (○—○), 6- (■—■) and 18- (▲—▲) month-old guinea pigs.

$^{45}Ca^{2+}$ accumulated either in the initial period or at the plateau phase. The non-specific calcium uptake (taken up by the K^+-loaded vesicles, 1.55 ± 0.12 nmol/mg protein) was similar in vesicles of all three age groups.

Na^+-dependent $^{45}Ca^{2+}$ uptake in the presence of various concentrations of $^{45}Ca^{2+}$. The difference observed in Na^+-dependent $^{45}Ca^{2+}$ uptake of the older guinea pigs was characterized further by studying the initial rate of $^{45}Ca^{2+}$ uptake. The $^{45}Ca^{2+}$ uptake was measured in the first 5 sec (the uptake of $^{45}Ca^{2+}$ appeared to be linear within this period), and the rate of $^{45}Ca^{2+}$ uptake was then calculated. The lower panel of Fig. 3 illustrates the initial rates of $^{45}Ca^{2+}$ uptake in the presence of external $^{45}Ca^{2+}$, ranging from 20 to 80 μM. The data show that there was a substantial reduction in the rate of $^{45}Ca^{2+}$ uptake in the vesicles from the older animal hearts (18 months). An Eadie–Hofstee plot of the data shown in the top panel of Fig. 3 demonstrates that the apparent maximum initial rate of $^{45}Ca^{2+}$ uptake (V_{max}) was reduced by 70% in the vesicles of the older animal hearts (20 ± 0.82 vs 54.2 ± 1.7 in 3 month olds). The K_m values were 58.4 ± 6.7 and 35.78 ± 1.14 μM for vesicles of 18-month- and 3-month-old guinea pig hearts respectively.

Na^+-dependent Ca^{2+} efflux. Calcium efflux was initiated afer 1 min of Na^+-dependent $^{45}Ca^{2+}$ uptake by increasing Na^+ concentration in the incubation medium. Figure 4 illustrates the time–course of $^{45}Ca^{2+}$ efflux for vesicles of three different age groups. The initial rate of Ca^{2+} efflux during the 5–10 sec was substantially lower in 18-month-old than in 3-month-old animals (0.11 ± 0.03 as compared to 0.25 ± 0.02 nmol · mg protein^{-1}, $P < 0.05$). When the efflux activity was evaluated as percent $^{45}Ca^{2+}$ retained in proportion to its basal value (Fig. 5), the initial efflux did not seem to differ. However, at the end of 120–180 sec of incubation, the vesicles from the older animal hearts retained 40% of the basal calcium, which was significantly higher than the vesicles isolated from 3-month-old animals, which retained only 21% of the basal $^{45}Ca^{2+}$, indicating that the Na^+-dependent $^{45}Ca^{2+}$ efflux was reduced in older animals. The $^{45}Ca^{2+}$ efflux in vesicles of 6-month-old guinea pigs was almost similar to the control vesicles (3 months) with a slightly greater retention of calcium.

DISCUSSION

The present data obtained from the anesthetized guinea pigs demonstrate that the contractile and relaxation functions of the left ventricle were impaired in the older animal hearts without any change in the systolic LVP pressure. *In vitro* studies have also revealed an impairment of these functions in aging rat papillary muscle [22]. In addition to the alteration in the left ventricular performance, the older guinea pigs also demonstrated a sinus bradycardia which may be due to the reduced sympathetic tone [23]. Since reduction in the heart rate was also demonstrated in non-anesthetized animals, it is unlikely that the observed bradycardia in the older animals was due to any differential age-related sensitivity to the anesthesia used. The lower heart rate in senescence has also been demonstrated in non-anesthetized rats [24]. When the older guinea pigs were treated with ouabain intravenously, the development of inotropic response was reduced but the appearance of ventricular arrhythmias was enhanced substantially when compared to the younger animals.

The Na^+-dependent $^{45}Ca^{2+}$ uptake also was reduced substantially in the vesicles of the older

animal hearts. The apparent maximal initial rate (V_{max}) declined by 70% with a significant reduction in the affinity for calcium. The passive diffusion of Ca^{2+} from the vesicles was not determined in the different age groups. However, the substantial reduction in the rate of $^{45}Ca^{2+}$ uptake and the affinity for Ca^{2+} in the vesicles of the older animal hearts were unlikely to be due to the differences in passive diffusion. The apparent rates of vesicular $^{45}Ca^{2+}$ efflux also were found to be significantly lower in older animals. In addition, the remaining Ca^{2+} in the vesicles after 2–3 min of efflux was significantly more in 18-month-old than in 3- to 6-month-old animals.

Na^+–Ca^{2+} exchange is considered to be an intermediate step between enzyme inhibition and the pharmacological response to cardiac glycosides. The current evidence of reduction of Na^+–Ca^{2+} exchange in the older animal hearts may suggest an impairment of the calcium transport in and out of the cell. At low concentrations of cardiac glycosides, only a fraction of the Na^+ pumping sites become inhibited and $[Na^+]_i$ rises in the close proximity of the inhibited sites, which in turn decreases the efficiency of the Na^+-linked Ca^{2+} extrusion mechanism. The increased $[Ca^{2+}]_i$ is captured by sarcoplasmic reticulum (SR) and released during successive beats, thus giving larger Ca^{2+} and tension transient (positive inotropy). When, because of the greater inhibition of the Na^+–K^+ pump with toxic concentrations of cardiac glycosides, there is further reduction of Na^+-linked Ca^{2+} extrusion, the $[Ca^{2+}]_i$ and the resting tension begin to increase. There is also an excessive uptake of Ca^{2+} by the mitochondria, leading to ultrastructural damage and reduced production of ATP [25]. This in turn can lead to reduced Ca^{2+} pump activities. The large increases in $[Ca^{2+}]_i$ under these conditions can give rise to after-depolarization [14] and to ectopic electrical activation of the myocardium.

In the present study, the membrane vesicles employed were largely right-side out vesicles (>70%). Since the activities of Na^+–Ca^{2+} exchanger were found to be significantly lower in older animals and since Na^+-dependent efflux of Ca^{2+} is important in the regulation of $[Ca^{2+}]_i$ [17], it may be justified to propose that older animals may have a higher tendency to develop cellular Ca^{2+} overload than younger animals (3–6 months). This concept is further supported by the observation that the vesicles from older animal hearts retained significantly higher Ca^{2+} even after several minutes of the inhibition of Na^+-dependent efflux. In the presence of toxic concentrations of cardiac glycosides, a further reduction of the Na^+-linked Ca^{2+} extrusion mechanism may thus make older animals much more susceptible to cellular Ca^{2+} overload, ectopic beats and arrhythmias than 3- to 6-month-olds. Na^+-dependent Ca^{2+} influx was also found to be lower in vesicles from the older animal hearts. The significance of this observation in the manifestation of cardiac glycoside toxicity is not clear to us. However, this may play a significant role in reduced inotropic response observed in the older animals, which would mean that in the presence of a therapeutic concentration of digitalis the Na^+-dependent Ca^{2+} influx may be more important, and this has been demonstrated to be lower in older animals. Mechanisms other than Na^+–Ca^{2+} exchange may also contribute to the altered sensitivity of the myocardium to digitalis. Studies indicate that the reduction of transmembrane calcium influx via voltage-gated calcium channels protects the heart against digitalis-induced arrhythmias [10, 11]. On the other hand, enhancement of Ca^{2+} influx through these channels by BAY K-8644 potentiates ouabain toxicity [36]. These reports may indicate a significant role of Ca^{2+} influx (and the elevation of cytoplasmic calcium) through the voltage-gated calcium channels in digitalis toxicity. Our recent studies in rats have demonstrated that the aging hearts are more sensitive to the calcium agonist BAY K-8644, suggesting that the voltage-gated calcium channels could be modified in the process of aging.* The increased calcium influx through the voltage-gated calcium channels may contribute to the development of calcium overload and may be an additional factor contributing to the increased sensitivity to digitalis.

In conclusion, the present study demonstrated that the older animal hearts are more sensitive to digitalis-induced arrhythmias. The increased arrhythmogenic toxicity of aging hearts to digitalis may partially be explained by the altered Na^+–Ca^{2+} exchange activity. However, other possible factors, namely the role of release of catecholamines during ouabain administration and the alterations in sarcolemmal calcium transport through mechanisms other than Na^+–Ca^{2+} exchange, need further investigation.

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