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# CARDIOTOXIC AND INOTROPIC EFFECTS OF OUABAIN ON ATRIA ISOLATED FROM RABBITS OF DIFFERENT AGES

NOBORU TODA

Department of Pharmacology, Shiga University of Medical Sciences, Seta, Ohtsu 520-21, Japan

**1** In sino-atrial node-right atrial preparations and left atrial preparations obtained from rabbits of different ages (2 to 360 days old), cardiotoxic and inotropic effects of ouabain were compared. Tachyarrhythmias were produced in immature and mature rabbit right atria exposed for 6 to 8 min to ouabain ($5 \times 10^{-6}$ M). The total number of contractions before the arrhythmias developed varied inversely with age (2 to 90 days old). Percentage reductions in the resting membrane potential, overshoot and maximum rate of rise induced by ouabain were greater in mature rabbit atria than in immature rabbit atria.

**2** Reduction of $Ca^{2+}$ in the bathing media suppressed the development of tachyarrhythmias.

**3** In electrically-driven left atria isolated from immature and mature rabbits, similar frequency-contractile force curves were obtained, maximum contractions being attained at a frequency of 120/min. Ouabain at $2 \times 10^{-7}$ M shifted the curve upward and at $10^{-6}$ M greatly potentiated the contractile force developed at low frequencies (6 to 30/min). Increase in contractions induced by ouabain relative to pre-drug contractions at a frequency of 60/min were greater in immature rabbit atria (15 days old) but the positive inotropic effect of $2 \times 10^{-7}$ M ouabain relative to the effect seen at $10^{-6}$ M was appreciably less.

**4** It may be concluded that atria isolated from immature rabbits tolerate higher concentrations of ouabain than those isolated from mature rabbits.

## Introduction

Cardiotoxic and inotropic effects of cardiac glycosides vary with age. Halloran, Schimoff, Nicholas & Talner (1970), Rogers, Willerson, Goldblatt & Smith (1972), Hayes, Butler & Cersony (1973) and Kelliher & Roberts (1976) have shown that neonatal and infant mammals tolerate higher doses of digitalis than adults but contradictory results have been described by Haag & Corbell (1940) and Marini, Sereni & Bottino (1962). In experiments *in situ*, the cardiotoxicity is associated not only with the direct action of digitalis on cardiac muscles but also with actions on a variety of regulatory mechanisms, such as those relating to the autonomic nervous system (Kelliher & Roberts, 1976) and hormones, as well as with the metabolism and distribution of applied digitalis (Glantz, Kernoff & Goldman, 1976). Isolated, perfused hearts of young guinea-pigs (20 to 25 days old) are reportedly less susceptible to ouabain than those of older guinea-pigs (Wollenberger, Jehl & Karsh, 1953). However, little information is available concerning systematic comparisons of the direct effect of cardiac glycosides on the myocardium obtained from mammals of different ages.

The present study was undertaken to clarify differences in the effect of ouabain on the rate or rhythm and the membrane potential of atrial muscles isolated from rabbits of different ages and to compare the involvement of $Ca^{2+}$ in the genesis of ouabain-induced toxicity in immature and mature rabbit atria. The positive inotropic effect of ouabain was also compared in electrically-driven left atrial preparations.

## Methods

Albino rabbits of either sex and different ages were anaesthetized with ether and killed by bleeding from the common carotid arteries. The heart was rapidly removed, and the sino-atrial (S-A) node-right atrial preparation and the left atrial preparation were prepared. The preparations were fixed horizontally between hooks, endocardial surface uppermost, in the muscle bath containing the nutrient solution which was maintained at $30 \pm 0.5$°C and was gassed with a mixture of 95% $O_2$ and 5% $CO_2$. The hook fixing the atrial appendage was connected to the lever of a force-displacement transducer (Nihonkoden Kogyo Co., Tokyo, Japan). The resting tension of S-A node-right atrial preparations was adjusted to 50 to 100 mg in atria from neonatal rabbits (2 to 5 days old), 100 to

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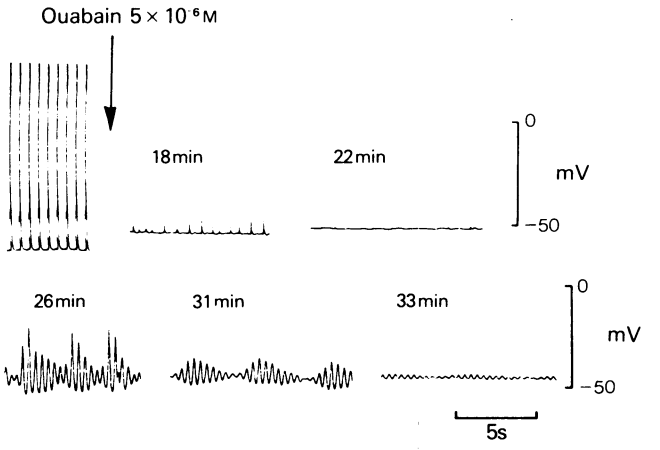


**Figure 1** Effects of ouabain on the action potential recorded from an atrial cell (up to 22 min) and an S-A nodal cell (26, 31 and 33 min) of the atrium isolated from a rabbit (day 90). After atrial action potentials disappeared, oscillatory potentials were recorded from an S-A nodal cell.

200 mg in atria from young rabbits (10 to 30 days) and 300 to 400 mg in atria from mature rabbits (90 days or older) (Toda, 1980). The cut end of the left atrial preparations was fixed by a pair of hooks which were connected to the electronic stimulator (Nihonkoden Kogyo Co.). Constituents of the solution were as follows (mM): $Na^+$ 162.1, $K^+$ 5.4, $Ca^{2+}$ 2.2, $Mg^{2+}$ 1.0, $Cl^-$ 159.0, $HCO_3^-$ 14.9, and dextrose 5.6. Before the start of experiments, the preparations were allowed to equilibrate for 60 to 90 min in the bathing media during which time the fluids were replaced every 10 to 15 min.

Neonatal rabbits housed in this laboratory ingested maternal milk and then artificial food for 360 days or longer. Rabbits of $2 \pm 1$ days ($61.2 \pm 3.7$ g body weight, $n = 10$), $5 \pm 1$ days ($89.6 \pm 11.1$ g, $n = 9$), $10 \pm 2$ days ($136.7 \pm 10.2$ g, $n = 7$), $30 \pm 3$ days ($655 \pm 51.2$ g, $n = 16$), $90 \pm 20$ days ($1892 \pm 218$ g, $n = 9$), $180 \pm 30$ days ($3922 \pm 151$ g, $n = 10$) and $360 \pm 60$ days after birth ($4080 \pm 221$ g, $n = 8$) were used; 15 day rabbits for experiments with left atria were 10 to 20 days old.

Intracellular recordings were made by use of microelectrodes with resistances of 10 to 30 megohms. Transmembrane potentials were recorded from single cells of the atrium and the S-A node. The membrane potential was recorded from a VC-9 oscilloscope (Nihonkoden Kogyo Co.) on films moving at speeds of 5 and 10 cm/s. Action potentials were recorded simultaneously from the S-A node and right atrium when necessary. Parameters of action potentials measured were the resting potential, overshoot, 10% and 90% durations, defined as the duration at the level of 10% and 90% the magnitude of action potentials, maximum rate of rise ($\dot{V}_{max}$) and cycle length between action potentials. Action potentials were recorded from different cells of the right atrium (2 to 10 penetrations) before the addition of ouabain, after $10 \pm 2$ min exposure to ouabain in control media and after $15 \pm 3$ min exposure to ouabain in $Ca^{2+}$-deficient media. Preparations were equilibrated for 30 min in experimental solutions deprived of $Ca^{2+}$ (1/10 normal $Ca^{2+}$). Both the membrane potential and contraction were also displayed on an ink-writing oscillograph (Sanei Sokki Co., Tokyo, Japan).

Left atrial preparations were stimulated by a train of 3 ms square pulses of supramaximum intensity (3 times threshold intensity) at a basic frequency of 60/min. The atria were stretched stepwise to attain the optimum length of muscles at which the maximum contraction was obtained. Experiments were carried out at the optimum length. The cross sectional area could not be measured because the left atrial preparations were fixed at 3 points, one hook connected to a force-displacement transducer and a pair of stimulating electrodes. Frequency-contractile force curves were obtained by raising the driving frequency stepwise from 6 to 180/min (Toda, 1969). The frequency-force curves were recorded before the addition of ouabain and after 30 min exposure to the glycoside.

Results shown in the text, figures and tables are expressed as mean values $\pm$ s.e. mean. Statistical analyses were made by use of Student's $t$ test. Drugs used were ouabain octahydrate (Sigma) and (±)-propranolol hydrochloride.

## Results

### *Cardiotoxic effect of ouabain*

The addition of ouabain in a concentration of $5 \times 10^{-6}$ M to right atria isolated from rabbits of

**Table 1** Modification by ouabain ($5 \times 10^{-6}$ M) of the rate, rhythm and activity of right atria or S-A nodes obtained from rabbits of different ages

| *Age* (days) | n | *Atrial rate* (beats/min) | *Time to arrhythmia* (min) | *Time to atrial arrest* (min) | *Time to S-A nodal arrest* (min) | *S-A nodal-atrial dissociation* (min) | *Total beats*[a] |
|---|---|---|---|---|---|---|---|
| 2 | 11 | 165 ± 9.2 | 8.0 ± 1.5 | 30.2 ± 1.4 | 33.5 ± 2.8 | 3.8 ± 1.8 (6)[b] | 1328 ± 274 |
| 5 | 13 | 156 ± 8.5 | 7.2 ± 1.3 | 32.5 ± 1.6 | 36.7 ± 3.1 | 5.3 ± 2.0 (9) | 1180 ± 271 |
| 10 | 8 | 136 ± 12 | 8.1 ± 1.5 | 27.8 ± 2.0 | 32.9 ± 2.9 | 5.1 ± 2.2 (7) | 1146 ± 256 |
| 30 | 13 | 132 ± 7.3† | 8.2 ± 0.9 | 32.1 ± 3.3 | 39.1 ± 4.1 | 8.6 ± 2.1 (9) | 1068 ± 129 |
| 90 | 11 | 97.4 ± 7.6** | 6.8 ± 0.9 | 29.4 ± 2.2 | 42.6 ± 3.4 | 13.5 ± 3.6† (8) | 658 ± 96† |
| 180 | 10 | 94.3 ± 5.9** | 6.1 ± 0.7 | 24.3 ± 1.7 | 37.0 ± 2.9 | 12.6 ± 2.0* (8) | 579 ± 54†† |
| 360 | 8 | 88.3 ± 7.5** | 6.5 ± 0.7 | 27.3 ± 2.7 | 34.8 ± 5.3 | 11.4 ± 2.5† (5) | 567 ± 53† |

N, number of preparations used. [a]Total beats until arrhythmias developed; [b] Number of preparations used for the observation. Significantly different from values obtained in atria from rabbits at day 2; * $P < 0.01$; ** $P < 0.001$; † $P < 0.05$; †† $P < 0.02$.

**Table 2** Modification by ouabain ($5 \times 10^{-6}$ M) of parameters of the membrane potential of right atria isolated from immature and mature rabbits

| | n | *RP* (mV) | *OS* (mV) | *10% D* (ms) | *90% D* (ms) | $\dot{V}_{max}$ (V/s) | *Cycle length* (ms) |
|---|---|---|---|---|---|---|---|
| *Day 2 to 10* | | | | | | | |
| Control | 200 (28) | 68.8 ± 0.4 | 14.3 ± 0.5 | 7.6 ± 0.3 | 76.9 ± 1.0 | 78.9 ± 1.9 | 397 ± 9.5 |
| Ouabain | 49 (9) | 56.1 ± 0.8** | −9.4 ± 1.3** | 5.4 ± 0.4** | 101 ± 16.1 | 29.0 ± 1.9** | 369 ± 11.9 |
| Δ | | −12.7 | −23.7 | −2.2 | +24.1 | −49.9 | −28 |
| | | (18.5%) | | (29.0%) | (31.3%) | (63.2%) | (7.1%) |
| *Day 90* | | | | | | | |
| Control | 156 (12) | 75.5 ± 0.5 | 20.5 ± 0.5 | 14.2 ± 0.4 | 134 ± 2.2 | 85.3 ± 2.7 | 577 ± 21.8 |
| Ouabain | 38 (7) | 56.6 ± 0.8** | −9.8 ± 1.5** | 11.3 ± 0.8* | 118 ± 4.8* | 20.2 ± 1.4** | 521 ± 41.2 |
| Δ | | −18.9 | −30.3 | −2.9 | −16 | −64.9 | −56 |
| | | (25.9%) | | (20.4%) | (11.9%) | (76.1%) | (9.7%) |

Action potentials were recorded from atrial appendage exposed to control media and for 8 to 12 min to ouabain. *n*, number of penetrations; figures in parentheses in *n* column represent the number of preparations used. RP = resting potential; OS = overshoot; 10% and 90% D = 10% and 90% durations; $\dot{V}_{max}$ = maximum rate of rise. Significantly different from control, * $P < 0.01$; ** $P < 0.001$.

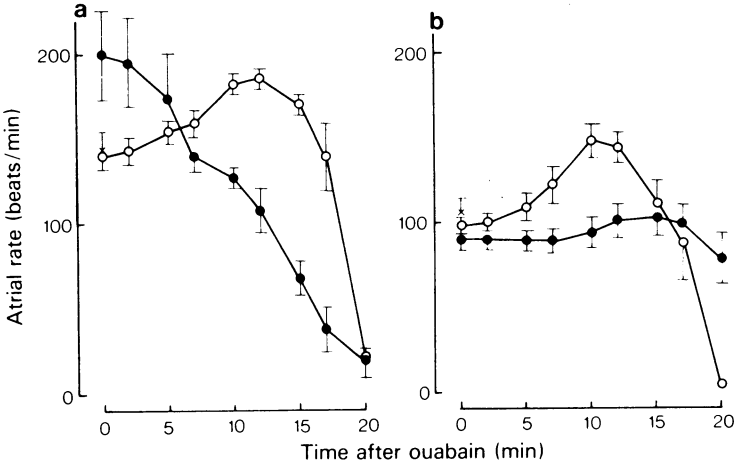


**Figure 2** Time course of the effect of ouabain ($5 \times 10^{-6}$ M) on the rate of atria exposed to normal (○) and $Ca^{2+}$-deficient (1/10 normal) media (●). (a) Immature rabbit atria (day 2 to 10); (b) mature rabbit atria (day 90). Crosses at 'zero' min represent the atrial rate before the bathing media were replaced with $Ca^{2+}$-deficient fluids. Vertical bars represent s.e. mean. In (a) $n = 13$ for normal $Ca^{2+}$ and $n = 8$ for 1/10 $Ca^{2+}$; in (b), $n = 11$ for normal $Ca^{2+}$ and $n = 9$ for 1/10 $Ca^{2+}$.

different ages caused a rapidly-developing arrhythmia and led to atrial arrest. The time course of alterations in the action potential of the right atrium and S-A node is presented in Figure 1. The S-A nodal rate was greater in atria from immature rabbits (2 to 10 days) than in those from mature rabbits (60 to 360 days). Average times required to induce the arrhythmia (development of irregular rhythm or increase of more than 10% in the rate) and the abolition of atrial and S-A nodal electrical activity did not significantly differ in mature and immature rabbit atria (Table 1). However, the total number of atrial contractions before the arrhythmia developed, decreased with increasing age; the values in atria at day 90 or older were significantly less than the values at day 2. In 3 out of 6 atria at day 2, the S-A nodal electrical activity persisted after atrial mechanical and electrical activities were abolished, while in the remaining 3, electrical activity was not recorded from the S-A node when the atrial activity disappeared. Incidence of such an apparent coincidence of S-A nodal and atrial electrical quiescence decreased with age. The period of dissociation of the S-A nodal and atrial electrical activities was related directly to age in rabbits from 2 to 90 days (Table 1). In preparations in which the atrial activity was abolished, electrical stimulation (up to 10 times threshold intensity) at a frequency of 60/min restored the activity which persisted for 5 to 15 min.

Parameters of the transmembrane potential recorded from control and ouabain ($5 \times 10^{-6}$ M)-treated atria obtained from mature (90 days) and immature rabbits (2 to 10 days) were compared (Table 2). Ouabain, after inducing marked arrhythmias, reduced the resting membrane potential, overshoot, 10% duration and $\dot{V}_{max}$. The 90% duration was shortened in mature rabbit atria but in contrast, was prolonged in immature rabbit atria in which the control value was appreciably smaller than the control value in mature rabbit atria. The percentage reduction in these parameters was greater in mature rabbit atria. When the atrial electrical activity disappeared, the resting membrane potential averaged 54.2 ± 2.1 mV (16 penetrations) in 6 immature atria and 58.9 ± 1.4 mV (30 penetrations) in 6 mature rabbit atria.

The time course of alterations in the average atrial rate induced by $5 \times 10^{-6}$ M ouabain is shown in Figure 2. In both mature and immature rabbit atria, the rate increased, the maximum increase being attained after approx. 10 min exposure to the drug. In atria exposed to $Ca^{2+}$-deficient media (1/10 the normal $Ca^{2+}$), the ouabain-induced tachycardia was abolished. In immature rabbit atria exposed to solutions deprived of $Ca^{2+}$, ouabain produced a time-dependent decrease in the rate until atrial mechanical arrest was obtained 24.5 ± 4.0 min ($n = 8$) later, a time similar to that seen in control media (27.0 ± 1.3 min, $n = 13$). In mature rabbit atria exposed to $Ca^{2+}$-deficient media, the atrial arrest was prolonged to 27.9 ± 2.0 min ($n = 9$) from the value of 23.3 ± 2.1 min ($n = 11$) in control media.

Reduction of $Ca^{2+}$ in the bathing media decreased the resting potential, overshoot and $\dot{V}_{max}$ and prolonged the action potential duration. In the $Ca^{2+}$-deficient media, exposure for 15 ± 2 min to $5 \times 10^{-6}$ M

**Table 3** Modification by ouabain ($5 \times 10^{-6}$ M) of parameters of the membrane potential of immature and mature rabbit right atria exposed to $Ca^{2+}$-deficient media

| | n | *RP* (mV) | *OS* (mV) | *10% D* (ms) | *90% D* (ms) | $\dot{V}_{max}$ (V/s) | *Cycle length* (ms) |
|---|---|---|---|---|---|---|---|
| *Day 2 to 10* | | | | | | | |
| Control ($Ca^{2+}$ 1/10) | 69 (11) | 55.1 ± 0.9 | −3.6 ± 1.4 | 7.9 ± 0.7 | 60.2 ± 1.7 | 28.9 ± 3.2 | 368 ± 12.2 |
| Ouabain | 11 (4) | 55.8 ± 3.0 | −12.1 ± 4.2* | 8.2 ± 1.0 | 54.8 ± 2.7 | 19.8 ± 4.6 | 500 ± 21.5** |
| Δ | | +0.7 | −8.5 | +0.3 | −5.4 | −9.1 | +132 |
| | | (1.3%) | | (3.8%) | (9.0%) | (31.5%) | (35.9%) |
| *Day 90* | | | | | | | |
| Control ($Ca^{2+}$ 1/10) | 80 (10) | 62.8 ± 0.6 | 1.8 ± 0.7 | 22.4 ± 1.5 | 124 ± 3.1 | 40.3 ± 3.1 | 621 ± 34.2 |
| Ouabain | 61 (10) | 59.1 ± 0.6** | −6.6 ± 1.0** | 30.3 ± 1.0** | 126 ± 4.0 | 20.9 ± 1.2** | 687 ± 34.2 |
| Δ | | −3.7 | −8.4 | +7.9 | +2.0 | −19.4 | +66 |
| | | (5.9%) | | (35.3%) | (1.6%) | (48.1%) | (10.6%) |

Action potentials were recorded from the atrial appendage exposed to $Ca^{2+}$-deficient media in the absence (control) and presence (for 12 to 18 min) of ouabain. *n*, number of penetrations; figures in parentheses in *n* column represent the number of preparations used. RP, OS, 10% and 90% D, $\dot{V}_{max}$ as in footnote for Table 2. Significantly different from control, *$P < 0.05$; **$P < 0.001$.

**Table 4** Positive inotropic effects of ouabain in electrically-driven left atria isolated from rabbits of different ages

| *Age* (days) | n | *Control* (× 10 mg) | *Ouabain* $2 \times 10^{-7}$ M (× 10 mg) | % *Increase* (A) | n | *Control* (× 10 mg) | *Ouabain* $10^{-6}$ M (× 10 mg) | % *Increase* (B) | *A/B* (%) |
|---|---|---|---|---|---|---|---|---|---|
| 15 | 6 | 24.6 ± 5.5 | 35.5 ± 6.6 | 49.2 ± 7.3 | 7 | 19.9 ± 3.3 | 59.3 ± 8.6 | 285.4 ± 102.1 | 17.2 |
| 30 | 5 | 180.0 ± 38.8* | 206.4 ± 38.1** | 19.0 ± 6.6† | 5 | 146.4 ± 37.0* | 223.8 ± 43.7* | 76.6 ± 43.1 | 24.8 |
| 90 | 7 | 269.9 ± 63.3* | 302.1 ± 63.0* | 19.1 ± 6.8† | 8 | 214.2 ± 47.1* | 328.4 ± 54.3** | 63.9 ± 7.8 | 30.0 |
| 180 | 8 | 255.5 ± 29.9** | 309.0 ± 36.2** | 22.0 ± 4.1* | 8 | 242.7 ± 40.6** | 358.9 ± 37.0** | 66.5 ± 21.3 | 33.1 |
| 360 | 5 | 276.6 ± 82.9* | 324.0 ± 89.5* | 18.0 ± 7.3† | 5 | 219.2 ± 62.9* | 327.8 ± 99.6* | 41.2 ± 19.1 | 43.7 |

The atria were driven at a frequency of 60/min. *n*, number of preparations used. Significantly different from values obtained in atria from rabbit at day 15, * $P < 0.01$; ** $P < 0.001$; † $P < 0.02$.

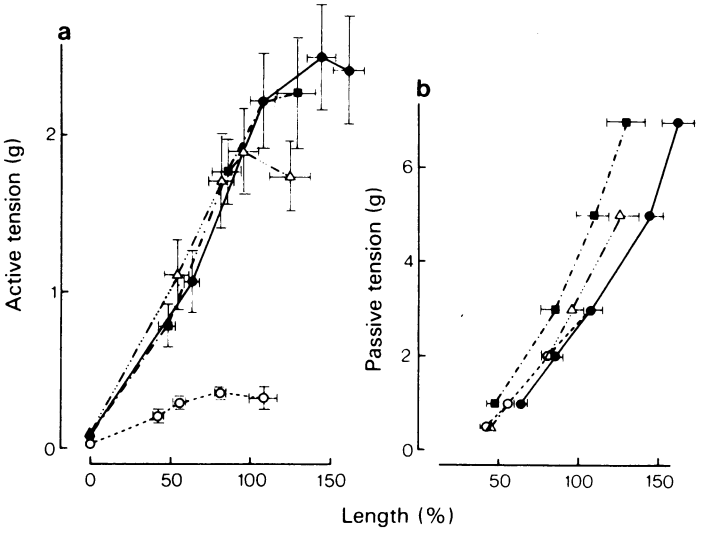


**Figure 3** Relationship between the length and the active (a) or passive (b) tensions of electrically-driven (60/min) left atria isolated from rabbits at day 15 (○), 30 (△), 90 (●) and 180 (■). The data were obtained from 4 to 6 preparations.

ouabain prolonged the cycle length and action potential duration and reduced the resting potential, overshoot and $\dot{V}_{max}$ in mature rabbit atria, while treatment with ouabain of immature rabbit atria prolonged the cycle length and reduced the overshoot (Table 3). The alterations were appreciably greater in mature rabbit atria.

*Inotropic effect of ouabain*

Left atria isolated from rabbits of different ages were stretched to give a 40 to 160% increase in length and passive and active tension developments were obtained. Maximum contractions were obtained at 80.8 ± 4.1% ($n$ = 8) increase in length in atria at day 15, 94.6 ± 9.7% ($n$ = 5) increase at day 30, 142.7 ± 8.8% ($n$ = 7) increase at day 90, 127.7 ± 11.7% ($n$ = 6) increase at day 180 (Figure 3) and 110% increase ($n$ = 2) at day 360.

The frequency of electrical driving stimulation was increased stepwise from 6 to 180/min. The contractile force of left atria increased with increasing frequencies in a range between 30 and 120/min in atria at day 15 and 90 (Figure 4). Treatment with $2 \times 10^{-7}$ M ouabain shifted the frequency-force curve upward. Increase in the concentration of ouabain to $10^{-6}$ M markedly potentiated the contractile force at low frequencies (6 to 30/min) in immature and mature rabbit atria (Figure 4). The results of experiments in which the positive inotropic effect of ouabain was studied in atria driven at a frequency of 60/min are summarized in Table 4. The percentage increase in contractile force was greater in atria at day 15 than in those at day 30 to 360. Increase in the ouabain concentration to $5 \times 10^{-6}$ M did not produce additional increase in contractions. Average increase in the contractile force induced by $2 \times 10^{-7}$ M ouabain relative to the maximum increase induced by $10^{-6}$ M ouabain was related directly to age (Table 4). When the effect of ouabain was stable, the addition of propranolol ($10^{-6}$ M) did not reduce the contraction.

## Discussion

Irregular rhythm and tachycardia developed in rabbit isolated right atria exposed to a toxic concentration ($5 \times 10^{-6}$ M) of ouabain; the time required for the development of the tachy-arrhythmias did not differ in atria isolated from immature and mature rabbits. It has been demonstrated that the effects of ouabain are dependent on the amount of activity of cardiac muscles after exposure of atria to the glycoside and not on the time of exposure (Moran, 1972). Myocardial ouabain uptake is greater in dogs with higher heart rate than with lower heart rate (Lloyd & Taylor, 1978). The present study revealed that the total number of contractions before the arrhythmias developed was related inversely to age, suggesting that the susceptibility of isolated atria to toxic concentrations of ouabain increases with age. Similar results were obtained in experiments *in situ* with rabbits of different ages, although the toxic effect is related not only to direct myocardial actions but also to actions on the autonomic nervous system (McLain, 1969; Roberts &

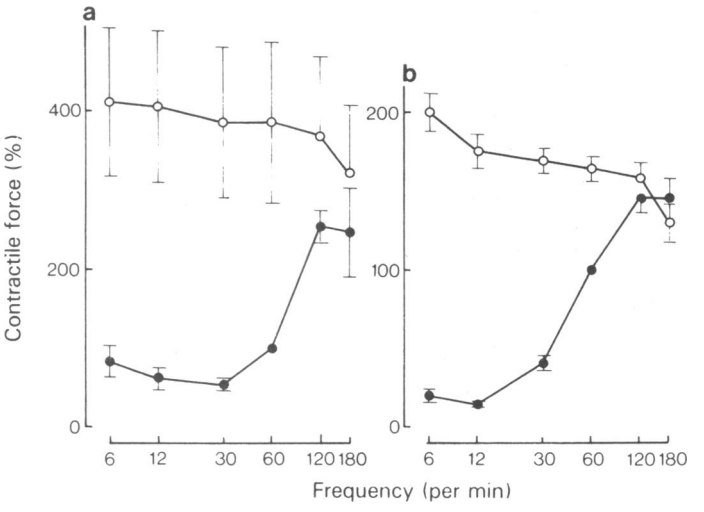


**Figure 4** Effects of ouabain on the frequency-contractile force curve of left atria isolated from rabbits at day 15 ($n = 7$) in (a) and 90 ($n = 8$) in (b). Preparations were exposed for 30 min to $10^{-6}$ M ouabain (○) before the frequency-force curve was obtained; control curve (●).

Kelliher, 1973; Gillis, Raines, Sohn, Levitt & Standaert, 1974; Gillis, Helke, Kellar & Quest, 1978). When arrhythmias developed, percentage reductions of the resting membrane potential, overshoot and $\dot{V}_{max}$ were greater in mature rabbit atria. Ouabain also causes a greater reduction of the maximal diastolic potential and $\dot{V}_{max}$ in adult dog Purkinje fibres than the reduction seen in neonate dog Purkinje fibres (Rosen, Hordof, Hodess, Verosky & Vulliemoz, 1975). Tissue ouabain uptake is higher in Purkinje fibres of young dogs (Rosen *et al.*, 1975) and human foetal cardiac tissues (Okita, Plotz & Davis, 1956) than in those of adult mammals. These results may indicate that S-A nodal, atrial and Purkinje cells of neonates are more resistant to ouabain than those of adults.

In mature rabbit atria, ouabain abolished the atrial electrical activity before the S-A nodal activity disappeared. Electrical driving restored the atrial activity, suggesting that the dissociation of S-A nodal and atrial activities is due to S-A nodal-atrial conduction block. Development of the conduction block was delayed in immature rabbit atria, probably by a smaller reduction of the resting potential, magnitude of action potential and $\dot{V}_{max}$. In the heart isolated from mature rabbits, ouabain has been shown to suppress electrical activity of atrial cells to a greater extent than those of S-A and A-V nodal cells (Toda & West, 1969).

Deprivation of $Ca^{2+}$ in the bathing media suppressed the tachycardia induced by ouabain in immature and mature rabbit atria, indicating that the involvement of $Ca^{2+}$ influxes in the genesis of ouabain-induced tachy-arrhythmias (Ferrier & Moe, 1973) is also valid in immature rabbit atria. Alterations in parameters of the membrane potential induced by ouabain were also reduced by deprivation of $Ca^{2+}$. Different time course of the rate in response to ouabain of immature and mature rabbit atria in $Ca^{2+}$-deficient media (Figure 2) may indicate that the direct suppressive effect of ouabain on the S-A nodal cell differs in these atria.

Increase in the contraction induced by $2 \times 10^{-7}$ M ouabain relative to the maximum increase induced by $10^{-6}$ M ouabain was related directly to age. Similar results were obtained in isolated papillary muscles (Boerth, 1975), although threshold concentrations of ouabain and those at which the maximum contraction was induced appreciably differed in atria (present study) and papillary muscles (Boerth, 1975). Such an age-related alteration is not due to reduced susceptibility of immature rabbit atria to ouabain, since the increase in the contractile force induced by the glycoside relative to the pre-drug contraction was greater in immature atria. Cardiac noradrenaline contents are related directly to age, and sympathetic nerve function matures during the early postnatal period (Friedman, Pool, Jacobowitz, Seagren & Braunwald, 1968; Schwieler, Douglas & Bouhuys, 1970; Friedman, 1972; Toda, Fu & Osumi, 1976). However, the involvement of noradrenaline released from adrenergic nerves in the positive inotropic effect of ouabain is excluded, since $\beta$-adrenoceptor antagonists and pretreatment of animals with reserpine do not reduce the effect of the glycoside (present study; Moran, 1972).

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