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Digitalis Toxicity and Baroceptor in Rabbits

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It is well-known that digitalis induces arrhyhmias associated with its toxicity. It has been reported that one of the mechanisms is direct stimulous action on myocardium and the other is indirect stimulous action through the autonomic nervous system. On the other hand, baroreflex plays a role to control autonomic nerve activity. Although digitalis seems to activate baroceptors and also denervation of baroceptors hastens digitalis toxicity, it is still unclear of dose-related change in baroceptor activity and baroceptor sensitivity *in vivo*.

Arterial blood pressure, cardiac rate, aortic nerve activity, renal sympathetic nerve activity were recorded simultaneously in anesthetized rabbits. The relationship between blood pressure and aortic nerve activity was figured using a X-Y recorder during a period of change in blood pressure induced by coarctation of the aorta. Ouabain in a dose of 30 $\mu g/kg$ was administered intravenously each 10 minutes until death. Aortic nerve activity increased by 78% and became more sensitive with the non-toxic dose of ouabain than the control level. However, aortic nerve activity and sensitivity decreased below the control level in larger dose than 60 $\mu g/kg$ which was compatible with toxic dose.

Thus, ouabain in therapeutic dose raised baroceptor activity by sensitization, whereas decreases in both sensitivity and activity were found in toxic dose. These findings indicate that the same effect as baroceptor denervation may be developed by digitalis toxicity.

Key Wards
Ouabain,
Digitalis toxicity,
Baroceptor,
Sensitization.

INTRODUCTION

Rational treatment generally begins with digitalis. Although the favorable hemodynamic effects of digitalis reduce congestive heart failure, this drug may provoke arrhythmias and conduction disturbances in as many as 20% of patients receiving this drug for treatment of heart failure (1). The two mechanisms have been reported about arrhythmogenesis. One is direct myocardial stimulation (2) and another is indirect stimulation through the adrenergic hyperactivity (3, 4).

As we and others have reported before (5, 6), digitalis has some effects on the autonomic nervous system. According to these reports, ouabain, a preparation of digitalis, induced biphasic change in sympathetic nerve activity. Before the appear-

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ance of arrhymias, ouabain induced suppression of sympathetic nerve activity which followed by arrhythmias and hyperexcitation of the activity. In these reports, it was concluded that digitalis stimulated the central nervous system (such as the vasomotor center) and sympathetic hyperactivity might play a role in digitalis arrhythmogenesis.

In the same study, furthermore, the diminution of lethal dose of ouabain and the shortening of survival period were observed following the removal of buffer nerves, whereas sympathetic nerve discharge was significantly increased. Since these results were not observed in intact animals, it was clarified that this defense mechanism, provided by buffer nerves, had the potent role to prevent fatal arrhythmias induced by digitalis toxicity.

Baroreflex has an action to stabilize arterial blood pressure by controlling sympathetic nerve activity (7), while there were several reports on the effects of digitalis on baroceptor activity (5, 8－11). They suggested that digitalis rendered the baroceptors hyperexcitable and thereby increased the afferent traffic to the medullary vasomotor centers. Although these results wereobtained by direct recording in cats (8) or dogs (9), *in vivo* (8) or *in vitro* (10), it has been reported that in these species, baroceptor afferentsof both the aortic and carotid sinus nerves, are mingled with chemoreceptor afferents (12, 13). These evidences indicate that effect of digitalis on baroceptors *in vivo* is still unclear. In addition, we are not aware of a report that mentioned about the grade of sensitization of baroceptors *in vivo* by direct recording with examination of the difference between before and after appearance of arrhythmias.

From these point of view, this study was designed to evaluate the relationship between ouabain and a baroceptor in greater detail. First purpose is to evaluate the magnitude of dose-response of baroceptor activity to ouabain especially in the respect of the occurrence of cardiac arrhythmias. Adult rabbits have an advantage to this purpose because their aortic nerve consists of barosensitive fibers alone (14). Second purpose is to evaluate the grade of sensitization of a baroceptor by ouabain. For this purpose, we developed a method to assess the grade of sensitization by recording the correlation between arterial blood pressure and aortic nerve activity.

MATERIALS AND METHODS

Adult rabbits of unselected as to sex and ranging from 2.5 to 3.0 kg in weight were used. They were anesthetized with urethane (600 mg／kg) and chloralose (50 mg／kg) administered intraperitoneally. After a tracheotomy was performed, each rabbit was ventilated on room air using a Harvard respirator pump. Gallamine (0.2 mg／kg, i. m.) was injected initially to prevent movement and was repeated during the course of the experiment as required. Blood gas was measured on occasion and kept within the physiological range with supplement of bicarbonate or oxygen as necessary.

1 . Surgical Procedures

The right femoral vein was cannulated for intravenous administration of drugs. A cannula was also introduced into the left lingual artery for recording arterial blood pressure or sampling arterial blood. This procedure permitted us to measure blood pressure and cardiac rate without harmful influence on cerebral blood flow. Blood pressure and electrocardiogram were used to monitor cardiac rhythm throughout the experiment.

2 . Recording of Nerve Activity

The left aortic nerve was exposed at the level of the larynx with fine forceps under a binocular microscope and placed on bipolar platinum electrodes. The left renal nerve was exposed retroperitoneally. The two electrical activities were recorded by a high gain amplifier simultaneously. Quantification of nerve activities were carried out using an integrator with a time constant of one second and compared with other parameters.

As other investigators (13) have reported, both the aortic and carotid sinus nerve in cats or dogs contain of not only baroceptor fibers but also chemoreceptor fibers. However, as Charlmers et al (14) reported, rabbits have few chemoreceptor fibers in their aortic nerves.

On the other hand, several reports (15) indicate that action on heart rate differs in the carotid sinus nerve and the aortic nerve. They insist that the stimulation of the aortic nerve induces bradycardia more significantly than the another. These evidences support the method of recording aortic nerve activity in rabbits for the present purpose.

3 . Correlation between Aortic Nerve Discharge and Arterial Blood Pressure

A left thoracotomy was performed and a snare was placed around the descending thoracic aorta (Fig. 1).

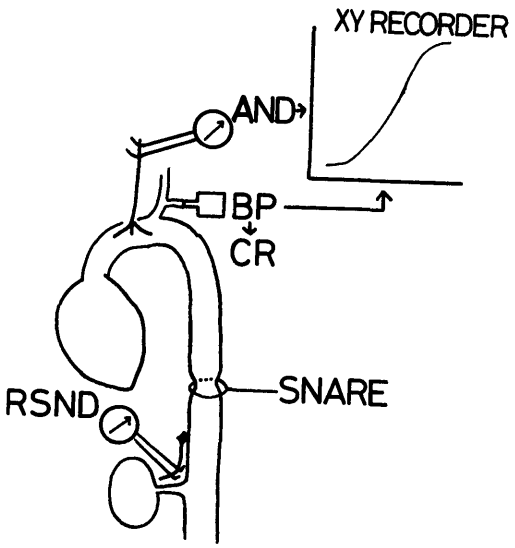


Figure 1. Schematic model of experiment. Aortic blood pressure (BP) is measured through a cannula inserted into the left lingual artery. Cardiac rate (CR) is counted by triggering the pulse. Aortic nerve discharge (AND) and renal sympathetic nerve discharge (RSND) are recorded. A snare is placed around the descending thoracic aorta to stimulate baroceptor by raising blood pressure. The correlation between BP and AND are figured using a XY recorder during the coarctation.

Coarctation of the aorta stimulated baroceptors by a raise of arterial blood pressure in the upper half of the body.

Correlation of arterial blood pressure and aortic nerve activity was figured and superimposed three times on each occasion using an X−Y recorder. This slope or shift was measured (16). The correlation was normalized by taking the center of both linear part of aortic nerve activity and blood pressure at 50 percent point. It becomes possible to examine the sensitivity of baroceptor *in vivo* with this method.

Irisawa and Ninomiya (16) reported the different response of baroceptor to given pressure between in pulsatile and non-pulsatile mode. It must be much relevant to examine the change in sensitivity of aortic nerve activity by fluctuation in blood pressure. From these point of view, we employed above method to investigate the response of baroceptor to given pressure in pulsatile mode *in vivo*.

4. Protocol for Ouabain Administration

After all parameters were stabilized and the control level was recorded, ouabain was administered intravenously in a single dose of 30 μg per kilogram of body weight as a bolus injection each 10 minutes until death. The relationship between blood pressure and aortic nerve discharge was recorded five minutes after each injection.

5. Data Analysis

The results are graphed in terms of mean ± S. E. M. Analysis of data was performed using Student's t test, ANOVA test (17) and linear regression with correlation coefficient. The criterion for statistical significance was $P<0.05$. Determination of arrhythmia in this experiment was settled as five ventricular premature beats in a minute.

6. Materials

The drugs used in this experiment were as in the following: Ouabain (Takeda Pharm. Co.), Gallamine (Teikoku Sangyo Co.), Urethane (Wako Chem. Co.), Chloralose (Wako Chem. Co.).

RESULT

1. Dose-Related Changes in Each Parameter Induced by Ouabain Administration

Figure 2 shows typical changes in cardiac rate, mean arterial blood pressure, aortic nerve discharge and renal sympathetic nerve discharge obtained from one experiment. After the first administration of ouabain in a dose of 30 μg/kg, aortic nerve discharge was elevated in parallel with arterial blood pressure. However, renal sympathetic nerve activity was decreased and bradycardia was developed.

The dose-response relationship for change in blood pressure, cardiac rate, aortic nerve discharge and renal sympathetic nerve discharge by ouabain administration in 8 rabbits are shown in Figure 3. Arbitrary

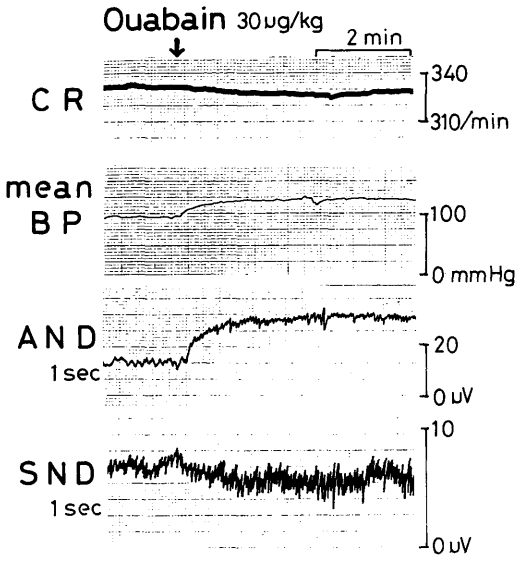


Figure 2. Typical changes in cardiac rate (CR), mean blood pressure (BP), mean aortic nerve discharge (AND) with time constant of 1 second, renal sympathetic nerve discharge (SND) with time constant of 1 second. At an arrow, ouabain in a dose of 30 μg/kg of body weight was administered intravenously as a bolus injection.

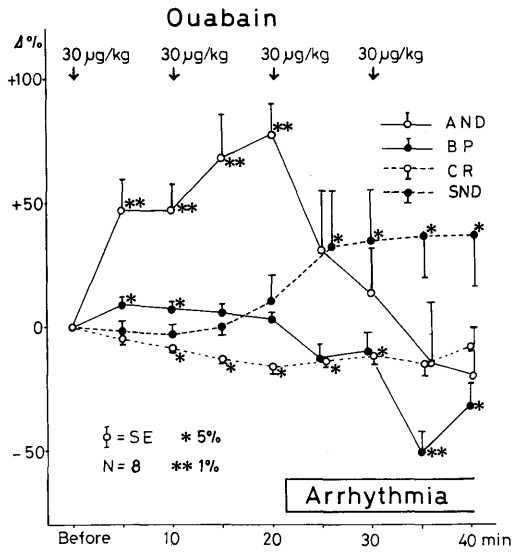


Figure 3. Statistical analysis of relative percent change of aortic nerve discharge (AND), blood pressure (BP), cardiac rate (CR) and renal sympathetic nerve discharge (SND) in 8 rabbits. Ouabain in a dose of 30 μg/kg is injected by each arrow. Following abbreviations are used. : SE (standard error of the mean), min (miniutes).

units of the ordinate are presented as relative percent change from each control value.

First administration of ouabain in a dose of 30 μg/kg induced significant increase in aortic nerve discharge and blood pressure by 46% and 8%, respectively On the other hand, cardiac rate and renal sympathetic nerve discharge decreased by 4% and 2%, respectively. The second injection (cumulative dose of 60 μg/kg) increased aortic nerve activity by 78% above the control level while blood pressure relatively decreased in comparison with the value of first injection. This phenomenon suggested that sensitization of baroceptor was developed, because the change in aortic nerve activity was not related to the change in blood pressure. In this phase, sympathetic nerve discharge increased slightly by 10% above the control but not significantly.

However, after the third injection (cumulative dose of 90 μg/kg), aortic nerve discharge and also blood

pressure started to decrease, while sympathetic nerve discharge increased significantly by 34%. This phase was coincided with the initiation of arrhythmias. Four out of 8 rabbits died after the fourth injection (cumulative dose of 120 μg/kg).

2. Correlation between Aortic Nerve Activity and Arterial Blood Pressure

To investigate the sensitization of aortic baroceptor furthermore, correlation between arterial blood pressure and aortic nerve discharge was observed. As shown in Figure 4, the curve was shifted to upper left with the cumulative dose of either 30 or 60 μg/kg in comparison with the control. But, after injection of 90 μg/kg which was compatible dose to develop ventricular arrhythmias, the inclination became dull and the peak value stayed below the peak of the control in spite of the higher level of the nerve activity at low pressure (left part of the curve). This suggests that ouabain in toxic dose desensitizes the responseof baroceptor to arterial blood pressure.

The statistical results of shifts in five rabbits were summarized in Figure 5. This figure depicts relative change from the control after administration of each cumulative dose of ouabain. The center of peak aortic nerve activity of the control was taken as 50 percent point and relative percent change was calculated. Regression analysis plus ANOVA test on slopes and y intercepts were compared.

In a dose of 30 μg/kg, the line was shifted to upper left and the slope became steeper ($Y=1.13X+2.3$) in contrast with the control ($Y=X$). After second injection of ouabain (cumulative dose of 60 μg/kg), the line shifted to further upper left, but slope was the approximately same as the control ($Y=0.95X+$

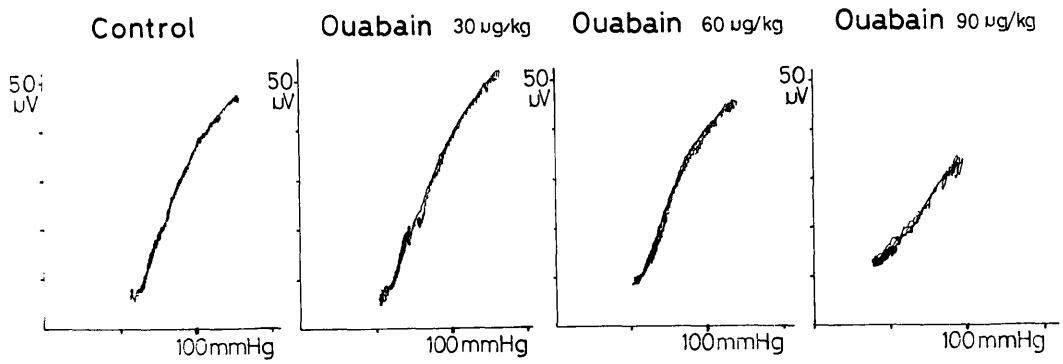


Figure 4. Typical changes in the relationship between mean aortic nerve discharge (ordinate) and aortic blood pressure (abscissa). From left to right, figure depicts the control, ouabain in a cumulative does of 30 μg/kg, 60 μg/kg and 90 μg/kg.

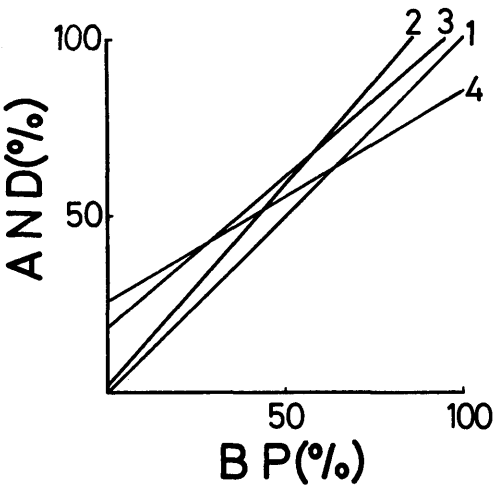


Figure 5. Statistical analysis of the relationship between aortic nerve discharge (AND) and aortic blood pressure (BP) in percent change obtained from 5 rabbits.
The regression equations are given in the text.
(1: control, 2: 30 μg/kg, 3: 60 μg/kg, 4: 90 μg/kg)

18.5). After the third injection (cumulative dose of 90 μg/kg), the base (y-intercept) of the line stayed still higher than the control but the slope became dull ($Y=0.59X+26$). Each line was different with each other significantly ($p<0.05$). It was impossible to examine the property in larger doses than 90 μg/kg because of fatal arrhythmias.

Thus, baroceptor became much sensitive to blood pressure under the ouabain administration to the cumulative dose of 60 μg/kg. However, in the dose larger than 60 μg/kg, the sensitivity started to decline and desensitization occurred.

DISCUSSION

These results indicate the different role of baroceptors in digitalis effects before and after appearance of toxicity. This suggests us to discuss on difference between effects of ouabain in therapeutic dose and those in toxic dose.

1. Ouabain in Therapeutic Dose

Other investigators have suggested that ouabain increases baroceptor activity by sensitization in cats (8), dogs (9), or rabbits (11). However, we are not aware of any studies which show the grade of sensitization of baroceptor activity in vitro by non-pulsatile stimulation with the statistical evaluation of baroceptor activity.

Quest and Gillis (10) examined the sensitization in isolated carotid sinus preparation of cats in non-pulsatile mode. Since they employed functional chemoreceptor elimination with acetic acid, it was not clear whether chemoreceptor fibers of the nerve was totally inactivated. Futhermore, since their study was made *in vitro*, it was impossible to know the relationship between arrhythmias and baroceptor activity.

From our results, in less dose than 60 μg/kg, ouabain induced increase in aortic nerve activity and blood pressure, and decrease in sympathetic nerve activity and cardiac rate. The elevation of aortic nerve activity was partly due to rise in arterial blood pressure, whereas ouabain has a vasoconstrictive effect on the excised artery in animals (18), and partly due to increase in the sensi-

tivity which was shown as a shift of relationship between aortic nerve activity and blood pressure.

Baroreflex has effects to control the autonomic nervous system (19). Rise in blood pressure decreases sympathetic nerve activity and increases vagal nerve activity by stimulation of baroceptors, and vice versa. In this point of veiw, these results suggest that the increase in baroceptor activity with ouabain in therapeutic dose decreased sympathetic nerve discharge and developed bradycardia (20).

2. Ouabain in Toxic Dose

Although, as far as we know, there are no detailed reports on changes in baroceptor activity induced by ouabain in toxic dose, ouabain in larger dose than 60 μg/kg developed toxicity with decrease in aortic nerve discharge and blood pressure in contrast with an increase in sympathetic nerve activity.

The decrease in aortic nerve discharge might be due to poor sensitivity of a baroceptor to blood pressure as well as a fall in blood pressure induced by poor contlactile force of myocardium because of arrhythmias (21).

As we (5) and others (6) reported, sympathetic hyperactivity, induced by stimulous action of digitalis on the central nervous system, must be one of the possible mechanisms of digitalis toxicity. They also reported that baroceptors had protective action to digitalis toxicity because denervation of baroceptors forced arrhythmias to appear earlier than in the intact. This phenomenon is due to the release of the central nervous system (vasomotor center) from the suppression induced by baroreflex. In this manner, denervation of baroceptors induced myocardium more sensitive to stimulation by increased sympathetic nerve activity.

These evidences suggest that overtherapeutic dose of ouabain may develop the toxication with increased sympathetic nerve activity by the desensitization of baroceptors. Thus, the poor response of a baroceptor to blood pressure may be called as chemical denervation.

Quest and Gillis explained the detailed mechanism concerning the sensitization of baroceptors (10). According to their report, ouabain can inhibit the active transport of K and Na by the membrane. Since inhibition of this transport system abolishes post-stimulated hyperpolarization of unmyelinated fibers and these reaction may further elevate the level of the membrane potential, it is concluded that these phenomena possibly lower the threshold level. Therefore, these effects easily enable the stimulus to come close to decreased threshold for a great number of fibers, those are resulted in increase of frequency in discharge.

On the other hand, if the level of the membrane potential rises further and beyond the threshold potential, poor response to given pressure (i.e., desensitization) may be developed. According to electrophysiology of the myocardium (22), ouabain elevates the resting level whereby membrane potential become close to threshold of activation. If the resting level rises higher than the threshold potential, the membrane

is not activated by a stimulation.

These phenomena are suggested by Figure 5 (90 μg/kg, at appearance of arrhythmias) which shows dullness of the slant and also a suppression in the peak value, in contrast to elevation of the level at low pressure (left half of the curve). Even under minimal pressure (leftest part of the curve), where nerve activity was always silent in intact animals, hyperactivity was developed because the fibers were still activated by sensitization. However, because the great number of fibers was inactivated by hyperpolarization, summation of activity became less. This mechanism can shift the curve higher at low pressure, and also make the slant dull as shown in Figure 5.

Thus, ouabain elevated aortic nerve activity as a result of increase in sensitivity to blood pressure in initial phase. Consequently, the toxicity of ouabain was suppressed easily in therapeutic dose. However, overdose of the drug developed poor response of baroceptor (chemical denervation). This phenomenon may participate in sympathetic hyperactivity in toxic dose and the development of arrhythmias.

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