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# EFFECTS OF OUABAIN AND CATECHOLAMINES ON THE REFRACTORY PERIODS AND CONTRACTIONS OF ISOLATED ATRIA

KIYOSHI MAEDA, KEIZO ITO, ATSUSHI SEKIYA AND ZENGO KANDA

*Department of Pharmacology, Nagoya University School of Medicine (Director: Prof. Zengo Kanda)*

ABSTRACT

Effects of ouabain and catecholamines and their modifications by propranolol were investigated on the functional refractory period (FRP), contractile force and heart rate in isolated atria of guinea-pigs and rats. Catecholamines produced a transient decrease in the FRP of guinea-pig atria, while ouabain caused a continual increase after a latent period of 5 to 10 minutes. The FRP in rat atria was increased by either ouabain or catecholamines. Pretreament with propranolol, which by itself produced a moderate increase in the FRP, completely blocked the effects of catecholamines, but did not prevent those of ouabain in both atria. It seems from these data on the FRP changes that no evidence was obtained for a ouabain-catecholamine relationship. However, from the observation on the spontaneously beating atria that the toxic effects of ouabain on contractility and rhythmicity were markedly reduced by propranolol it would be suggested that catecholamines might be related to the action of ouabain in its toxic stage.

## INTRODUCTION

Recently, interest has accumulated on the possibility that the action of cardiac glycosides might in some way have a correlation to endogenous catecholamines and sympathetic innervation. Méndez *et al.*[1] reported that in sympathectomized and adrenalectomized dogs acetyldigitoxin caused death by ventricular arrest instead of ventricular fibrillation and that the lethal dose of the glycoside was increased. Tanz[2] demonstrated that ouabain-induced augmentation of the cat papillary muscle was significantly attenuated by pretreatment with reserpine or the administration of dichloroisoproterenol (DCI), and suggested that the mechanism of the positive inotropic action of ouabain might be related in part to its ability to release endogenous catecholamines. Govier[3] noted the similarity of time course changes in the rabbit atrial refractory periods produced by ouabain and norepinephrine, and discussed the mechanism of action of ouabain with emphasis on the role of catecholamines.

Beta adrenergic blocking agents have been known to possess antifibrillatory

前田　清，伊藤敬三，関谷　淳，神田善吾
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properties. Lucchesi and Hardman[4] reported that DCI could reverese ouabain-induced arrhythmias in the dog. Sekiya and Vaughan Williams[5] compared the antifibrillatory actions and effects on intracellular cardiac potentials of pronethalol, disopyramide and quinidine with a suggestion of the possibility that some of the actions of pronethalol might not be directly related to its action in blocking beta-receptors. Evidence was also provided by Lucchesi[6] that pronethalol possessed an additional pharmacologic effect to account for its antiarrhythmic action. The newer compound, propranolol, introduced by Black *et al.*[7][8], has been well investigated and proved to be the more potent beta-receptor blocking agent and to have no such sympathomimetic effects in itself as DCI.

The present work was undertaken to examine changes in functional refractory periods and contractions induced by ouabain and catecholamines in guinea-pig and rat atria, and the effects of propranolol to modify these actions, in an effort to find an interaction between them.

## METHODS

Guinea-pigs weighing 500 to 700 g and rats of 160 to 240 g were killed by a blow at the base of the neck, and the heart rapidly removed. The atria were freed of ventricular muscle, connective tissue, fat and blood vessels, then suspended horizontally in a water-jacketed bath containing 50 ml of modified Ringer-Locke solution maintained at 31°C. Oxygenation of the bathing fluid with a mixture of 95% oxygen and 5% carbon dioxide was carried out with the apparatus described by Szekeres and Vaughan Williams[9]. The Ringer-Locke solution had the following composition in grams per liter: NaCl, 7.3; KCl, 0.42; $CaCl_2$, 0.24; $NaHCO_3$, 2.1; glucose, 0.2. The bicarbonate concentration employed maintained the pH at 7.4.

The tip of the left atrium was attached to a pair of silver stimulating electrodes; the atria were stimulated at a driving frequency of 240 beats per minute by suprathreshold, square wave pulses of 1 millisecond duration delivered by a Nihon Kohden MSE-3 stimulator. The functional refractory periods (FRP) were measured by applying a test stimulus from the second stimulator at increasing intervals following the fundamental driving stimuli until an electrographic response to the stimulus recorded from the appendage of the right atrium was observed on a beam of a Nihon Kohden VC-6 oscilloscope. Contractile force and heart rate were measured on the spontaneously beating atrial preparations of guinea-pigs by using a Shinkoh EN604L semi-conductor strain gauge and a Nihon Kohden RT-2 tachometer, respectively.

The preparations were equilibrated for 60 minutes before any drug administrations were made. Drug concentrations were expressed as grams of salt

per milliliter of bathing fluid. The drugs used were ouabain, norepinephrine, isoproterenol and propranolol (Sumitomo Chemical, Inderal).

## RESULTS

*1. Effects on contractile force and heart rate in the spontaneously beating atria of guinea-pigs.*

Figure 1 illustrates the effetcs of isoproterenol in a concentration of $10^{-7}$ on contractile force and heart rate and its blockade by prior treatment with propranolol, $5 \times 10^{-7}$, in typical experiments. Propranolol produced depression

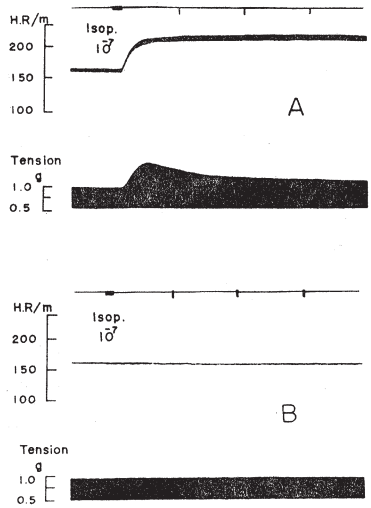


FIG. 1. Effects of isoproterenol on heart rate and contractile force of the spontaneously beating guinea-pig atria (upper set of tracings, A) and its blockade by propranolol, $5 \times 10^{-7}$ (lower set of tracings, B). Time marks, 2 min.

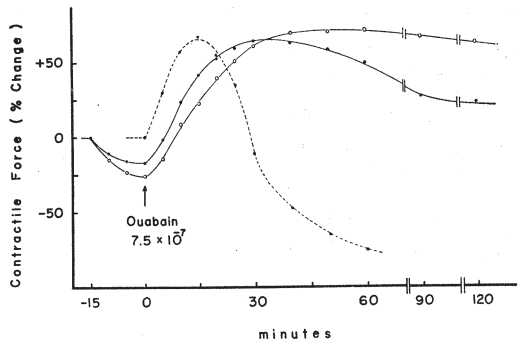


FIG. 2. Effects of ouabain on contractile force of the spontaneously beating guinea-pig atria pretreated with propranolol, $5 \times 10^{-7}$ (filled circles, solid line) and $10^{-6}$ (empty circles). Filled circles, broken line; controls of ouabain alone.

of contractile force and the slowing of heart rate roughly proportional to the dose; in a concentration of $5 \times 10^{-7}$, contractions and heart rate were reduced by 15% and 10%, respectively, at 15 minutes after the administration.

As shown in figure 2, ouabain in a toxic concentration of $7.5 \times 10^{-7}$ induced augmentation in contractile force, a maximum of which was reached at 15 minutes and followed by reduction of amplitude and occurrence of atrial tachycardia and fibrillation. By 40 minutes amplitude decreased to one half of the initial value. Pretreatment with propranolol at 15 minutes before the administration of ouabain markedly prevented ouabain intoxication, while a similar degree of positive inotropic effects as in the controls of ouabain alone was ob-

tained and maintained during the 120- to 180- minute of observation.

*2. Effects on refractory periods in guinea-pig driven atria.*

After equilibrium has been reached, the mean value for the FRP in the 7 control guinea-pig atria was 125 with a standard error of ± 4 milliseconds, and this value did not change significantly during the course of experiment.

Isoproterenol in a larger dose of $10^{-7}$ added to the driven atria tended to induce spontaneous extrasystoles, making further measurements of the FRP impossible. Even in a concentration as low as $5 \times 10^{-9}$, isoproterenol had the ability to elicit moderate positive inotropic effects, but failed to produce measurable changes in the FRP. Figure 3 presents the % changes in the FRP caused by ouabain in the atria with or without propranolol pretreatment. Isoproterenol in a concentration of $5 \times 10^{-8}$ produced a decrease in the FRP. The decrease appeared at 3 minutes and reached a maximum of 116 ± 3 milliseconds at 6 minutes, and after that the FRP increased again to the initial level. Norepinephrine, $5 \times 10^{-7}$, produced the same effects, and these effects of catecholamines to decrease the FRP were completely blocked by pretreatment with propranolol, which by itself in concentrations larger than $10^{-6}$ caused a moderate increase in the FRP and in a concentration of $5 \times 10^{-7}$ mainly used in the present experiments a slight increase of 2 milliseconds at 15 minutes.

Ouabain, contrary to catecholamines, produced a continual increase in the FRP after a latent period of 5 to 10 minutes; the mean value at 30 minutes was 134 ± 4 milliseconds, showing a 7% increase of the initial value. Conduction

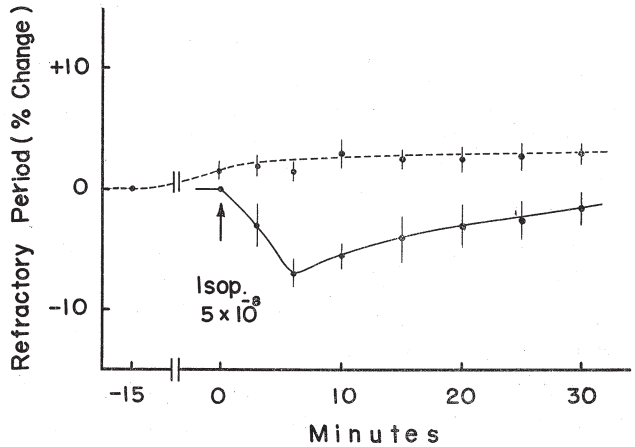


FIG. 3. Effects of isoproterenol on the FRP of guinea-pig atria with or without propranolol pretreatment. Solid line; without pretreatment. Broken line; with pretreatment. Vertical lines; standard errors.

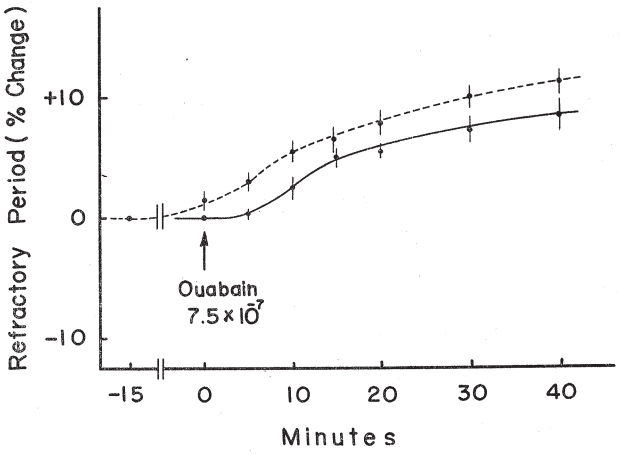


FIG. 4. Effects of ouabain on the FRP of guinea-pig atria with or without propranolol pretreatment. Solid line; without pretreatment. Broken line; with pretreatment.

disturbance, a toxic effect of ouabain in the electrically stimulated atria, was observed at between 30 and 40 minutes in three of the 9 atria. The response to ouabain in the FRP is illustrated in figure 4 in comparison with the results obtained in the atria pretreated with propranolol, which did not modify the effects of ouabain in the FRP, although occurrence of conduction disturbance was prevented in the atria pretreated.

*3. Effects on refractory periods in rat driven atria*

The mean initial FRP in 25 rat atria was 88 ± 3 milliseconds. Isoproterenol

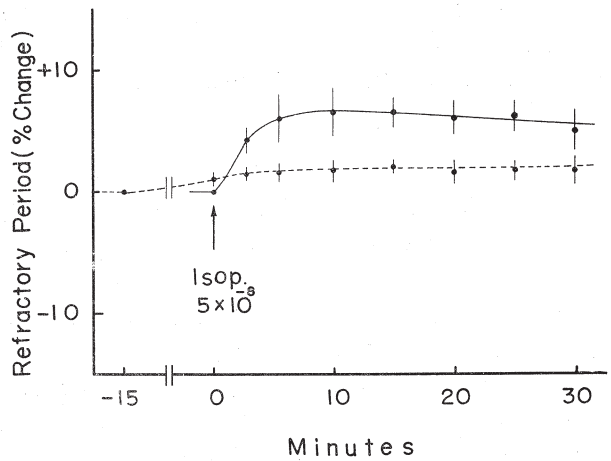


FIG. 5. Effects of isoproterenol on the FRP of rat atria with or without propranolol pretreatment. Solid line; without pretreatment. Broken line; with pretreatment.

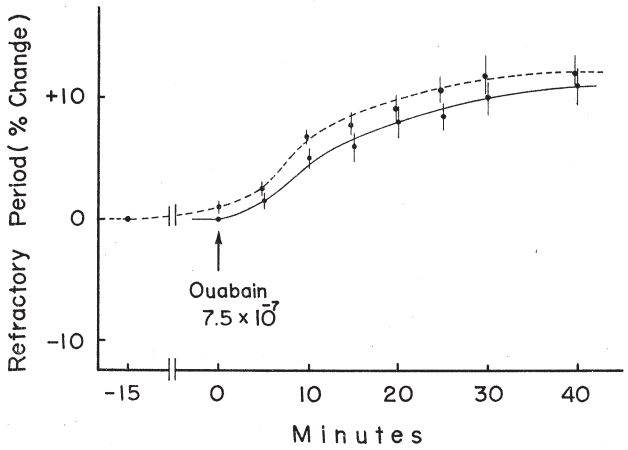


FIG. 6. Effects of ouabain on the FRP of rat atria with or without propranolol pretreatment. Solid line; without pretreatment. Broken line; with pretreatmant.

in concentrations of $5 \times 10^{-8}$ to $5 \times 10^{-7}$ increased the FRP to a value of $95 \pm 4$ milliseconds at 5 minutes, and after that this new level was not significantly altered.

As the rat is known to belong to species with lower sensitivity to cardiac glycosides, ouabain in a concentration of $5 \times 10^{-5}$ was employed in the present work. When the dose was added to bathing fluid, moderate augmentation in contractile force and a progressive increase in the FRP occurred after a shorter latent period of less than 5 minutes. As in the guinea-pig atria the effects of isoproterenol were markedly inhibited in the presence of propranolol, while ouabain-induced changes in the FRP were hardly modified by propranolol.

The results obtained on rat atria are presented in figures 5 and 6.

## DISCUSSION

With regard to the FRP changes caused by ouabain in isolated atria, the present data obtained on guinea-pigs are not consonant with the findings of Govier[3], who demonstrated that ouabain as well as norepinephrine produced in rabbit atria a marked decrease in the FRP, and suggested that the action of ouabain on the atrial refractory periods had two opposing components; a release of catecholamines and an intrinsic action of ouabain itself. Our data indicate that the FRP was decreased by the administration of isoproterenol and norepinephrine but increased by ouabain. Méndez and Méndez[10] observed in denervated hearts or heart-lung preparations of dogs that digitoxin and ouabain increased considerably the refractory periods of the auricular muscle.

Tanz[2] reported that the positive inotropic effects of ouabain were significantly reduced in the cat papillary muscles excised from the reserpine pretreated animals or in the preparations treated with an appropriate dose of DCI. While similar results in various preparations have been reported by some other investigators[11)12)13)], Yelnosky and Ervin[14] demonstrated that the ability of ouabain to increase the myocardial force of contractions was not inhibited in dogs pretreated with reserpine. Morrow *et al.*[15] reported that the inotropic and arrhythmic doses of ouabain were independent of autonomic innervation and myocardial catecholamine stores. The previous report from this laboratory[16] also indicated that ouabain produced as great an increase in contractile force and heart rate in the reserpinized guinea-pig atria as in the controls.

The effects of isoproterenol to decrease the FRP and to augment the contractile force of guinea-pig atria were completely blocked by propranolol pretreatment, whereas the effects of ouabain still remained in the presence of propranolol. Moreover, in rat atria, the FRP of which was increased by either catecholamines or ouabain, no modification of the effects of ouabain was induced by propranolol. Eventually from the present data on the FRP changes no evidence was obtained for a ouabain-catecholamine relationship.

The fact, however, that the toxic effects of ouabain on contractility and rhythmicity were definitely reduced by pretreatment with propranolol suggests that catecholamines might have a correlation to cardiac glycosides in their toxic stages.

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