POTENTIATION OF TOXICITY AND POSITIVE INOTROPIC EFFECT OF OUABAIN BY FUROSEMIDE IN GUINEA PIG HEART

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Abstract—The present study was undertaken to elucidate the potentiation by furosemide of toxicity and positive inotropic effect of ouabain in guinea pigs. Arrhythmogenic responses to ouabain as well as the lowering of its lethal dose were potentiated by pretreatment with furosemide in guinea pigs.

The potentiation of ouabain toxicity after furosemide administration was inhibited by pretreatment with potassium sparing diuretic, prorenocaste. Furosemide–induced potentiations of contractile force and arrhythmogenic effect of ouabain were also observed in isolated guinea pig papillary muscle. However after pretreatment with furosemide, ouabain produced arrhythmias without any significant changes in either left ventricular or subcellular fractions binding of 3H-ouabain in guinea pigs. These findings suggest that furosemide–induced potentiation of ouabain toxicity is at least in part associated with the decreased intracellular potassium content of guinea pig heart. It was also demonstrated that the positive inotropic action induced by subtoxic dose of ouabain was potentiated by furosemide in guinea pig papillary muscle preparation.

Key words: ouabain, furosemide, prorenocaste, automaticity, positive inotropic effect

INTRODUCTION

When digitalis administered to patients with congestive heart failure, the possibility of a drug interaction with furosemide, a strong diuretic should be expected to occur. Presently, little is known about the basic mechanisms that cause this interaction between digitalis and diuretics (Seller et al., 1975a, 1975b). In the past, investigations

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have been carried out using several different species (Akera et al., 1970; Okita et al., 1973; Schwartz et al., 1974). For this reason results have often been contradictory. These conflicting results may be attributed to experimental conditions; namely, difference in species (Schwartz et al., 1974) and dosage schedules. Because digitalis has a variety of cardiovascular effects on different species, it is a matter of considerable importance to use the same animal species throughout an experiment. No reports are available in the literature concerning the effect of combined administration of ouabain and furosemide both in vivo and in vitro on the guinea pig heart.

In line with above the present experiment has been designed to study the drug interaction between ouabain and furosemide in guinea pigs. Attention was focused on the following three points: changes in toxic and lethal doses induced by combined treatment with ouabain and furosemide; influence of furosemide on the contractile force and automaticity induced by ouabain in isolated papillary muscle; and effects of furosemide on 3H-ouabain distribution in cardiac muscle and in its subcellular fractions.

**METHODS**

1. *The toxic dose and lethal dose of ouabain in guinea pigs.*

Age matched male guinea pigs weighing approximately 520 g were used. Guinea pigs were anesthetized with a 1 g/kg i. p. dose of urethane and were divided into two groups. The first group was used as the control and a 5.2 $\mu$g/min (0.26 ml/min) dose of ouabain (Merek) was infused into the jugular vein through an indwelling venous catheter via constant infusion pump (LKB). The second group was subjected to combined administration of ouabain and furosemide which was administered at a dose of 2 mg/kg i. v. five minutes before ouabain administration. Second limb lead ECG was recorded using a direct writing electrocardiograph. Minimal dose which produced ventricular arrhythmias (multifocal ventricular premature beat, bigeminal rhythm or ventricular tachycardia) was regarded as a toxic dose of ouabain. The dose of ouabain which produced cardiac arrest preceded by ventricular fibrillation was regarded as the lethal dose of ouabain. The toxic dose and lethal dose of ouabain were expressed in terms of $\mu$g/kg of ouabain.

2. *The binding of tritium-labeled ouabain by cardiac muscle and by subcellular fractions isolated from guinea pig heart.*

A study was conducted to determine the significance of ouabain-binding to various structural components of cardiac muscle cell at the point of cardiac standstill. Tritium-labeled ouabain (New England Nuclear Co.) was infused into jugular vein of guinea pig under the same conditions as aforementioned. When cardiac arrest was induced by 3H-ouabain, heart was removed and immediately placed in ice-cold medium (2-4°C). Fractionation of subcellular components was accomplished by the procedure described by Auditore and Murray (1962). Namely, hearts were homogenized and homogenates were centrifuged to separate subcellular fractions (debris, mitochondria, microsome and supernatant fraction). Blood samples, heart muscles and subcellular fractions were
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ashed using a Sample Oxidizer (Packard). Concentration of \(^{3}H\)-ouabain in heart muscle, blood and subcellular fractions was calculated by counting radioactive \(^{3}H\)-ouabain in the sample. Concentration of \(^{3}H\)-ouabain in blood and supernatant fraction was expressed in terms of \(\mu g/ml\). Concentration of heart muscle and its subcellular fractions was expressed in terms of \(\mu g/g\) wet weight.

3. Isometric tension and automaticity in the isolated guinea pig papillary muscle.

Guinea pigs were sacrificed by means of carotid artery bleedings. Hearts were quickly removed and suspended in a constant temperature chamber at \(37\pm1^\circ C\) with modified Locke–Ringer’s solution. The modified Locke–Ringer’s solution was oxygenated with a 95% \(O_2\) and 5% \(CO_2\) gas mixture. Papillary muscles were isolated from right ventricle and suspended in organ bath which contained the modified Locke–Ringer’s solution. Using strain-gauge transducer (Schinkoh) via electric manometer (Nihon Kohden), the contractile force of papillary muscle was recorded isometrically on an ink–writing oscillograph (Nihon Kohden) (Gold and Cattel, 1940). Isolated papillary muscle obtained from guinea pig heart did not contract without electrical stimulation. Electric stimulation was produced by an Electrical Stimulator (Nihon Kohden) and Isolator (Nihon Kohden). Using a pair of platinum electrodes, papillary muscle preparations were driven by a rectangular current pulse (5 msec in duration, approximately 10% threshold) at a constant rate (1 Hz). After an equilibration of about 40 minutes, the perfusate was changed. Test compound solution was diluted with the solvent and deionized water and was added to the 30 ml organ bath at a volume of less than 0.3 ml. The final concentration of test compound was expressed in terms of \(g/ml\). The composition of modified Locke–Ringer’s solution was as follows (g/L): \(NaCl\) 9.0; \(KCl\) 0.42; \(CaCl_2\) 0.24; glucose 1.0 and \(NaHCO_3\) buffer (pH 7.2). The solution was aerated with 95% \(O_2\) and 5% \(CO_2\).

Data were analyzed by student’s t-test. \(P\) values less than 0.05 were considered statistically significant.

RESULTS

1. The toxic dose and lethal dose of ouabain in guinea pigs.

Using a constant infusion pump, a dose of 5.2 \(\mu g/ml\) ouabain infused into jugular vein through an indwelling venous catheter. Several different types of ventricular arrhythmias appeared during continuous administration of ouabain. Minimal dose which produced ventricular arrhythmia aforementioned was regarded as a toxic dose of ouabain. In this experiment, the toxic dose of ouabain was \(151.8\pm11.8 \mu g/kg\) (mean \(\pm\) SEM) in ouabain-treated control group. This value was also expressed in terms of 50.3% \(LD\) (Table 1). On the other hand, combined administration of ouabain and furosemide produced arrhythmia at a dose of \(124.4\pm5.6 \mu g/kg\) of ouabain. However, this difference between the toxic dose of ouabain treated control group and that of the combined drug administered group was not statistically significant. This potentiation of ouabain-induced arrhythmias after pretreatment with furosemide was antagonized by treatment with prorenoate, a potassium sparing diuretic (Table 1).
The lethal dose of ouabain-treated control group was 300.8±1.3μg/kg. After pretreatment with furosemide, the lethal dose of ouabain decreased 225.5±14.1μg/kg. Significant difference existed between ouabain–treated control group and combined drug administered group (P<0.01).

2. Concentration of \(^{3}H\)-ouabain in plasma, cardiac muscle and subcellular fraction in the isolated guinea pig heart at the end-point of cardiac arrest.

Concentration of \(^{3}H\)-ouabain in plasma in control group was 1.12±0.25μg/ml at the end-point of cardiac arrest (Table 2). Whereas in the furosemide pretreated group it was only 0.60±0.13μg/ml. No statistically significance was noted between control group and the group which was treated with ouabain plus furosemide. Under the same experimental conditions, concentration of \(^{3}H\)-ouabain in left ventricular myocardium in control group and furosemide pretreated group was 1.00±0.09 and 0.72±0.04μg/g wet weight respectively. Again, no significant difference was demonstrated between the two groups (Table 2).

**Table 2.** \(^{3}H\)-ouabain concentration in guinea pig blood and cardiac muscle at cardiac arrest

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Blood (μg/ml)</th>
<th>Cardiac muscle (μg/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouabain</td>
<td>9</td>
<td>1.12±0.25</td>
<td>1.00±0.09</td>
</tr>
<tr>
<td>Furosemide+Ouabain</td>
<td>3</td>
<td>0.60±0.13</td>
<td>0.72±0.04</td>
</tr>
</tbody>
</table>

Results indicate the mean±SEM.
N=the number of animal studied.

In the studies of ouabain distribution in subcellular fractions, \(^{3}H\)-ouabain binding in microsomal fractions of left ventricular myocardium was much greater than that in other fractions (Table 3). This increase in binding was statistically significant (P<0.01). On the other hand, in the control group there was increased binding of \(^{3}H\)-ouabain in plasma, cardiac muscle and microsomal fractions. That was proportional to the intravenous dose of \(^{3}H\)-ouabain (Table 3).

3. The contractile force and automaticity of isolated guinea pig papillary muscle.

With the addition of a dose of 10^{-7}g/ml ouabain into the organ bath, the contractile force, which was recorded as the change in phenylephrine-induced baseline tension, was significantly reduced in normal guinea pigs (Table 3).

**Table 1.** Toxic dose and lethal dose of ouabain in guinea pigs

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Toxic dose (μg/kg)</th>
<th>Lethal dose (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouabain</td>
<td>10</td>
<td>151.8±11.8</td>
<td>300.8±13.8</td>
</tr>
<tr>
<td>Furosemide+Ouabain</td>
<td>10</td>
<td>124.4±5.6</td>
<td>225.5±14.1*</td>
</tr>
<tr>
<td>Prorenoate+Ouabain</td>
<td>5</td>
<td>156.4±12.1</td>
<td>282.5±15.5</td>
</tr>
<tr>
<td>Prorenoate+Furosemide+Ouabain</td>
<td>6</td>
<td>146.9±9.2</td>
<td>238.8±14.6</td>
</tr>
</tbody>
</table>

Results indicate the mean±SEM.
* significantly different (P<0.01) from ouabain.
N=the number of animal studied.
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The force of isolated papillary muscle increased gradually with time. Maximal positive inotropic action induced by ouabain was obtained within approximately 10 minutes. As shown Fig. 1, ouabain increased the papillary muscle contractile force by 8.59 ± 2.66% as compared with that in the untreated control. On the other hand, it was demonstrated that after combined treatment with ouabain and furosemide the tension of papillary muscle increased by 21.48 ± 2.22% as compared with that in untreated control. Significant difference was observed between control group and furosemide plus ouabain treated group with respect to ouabain-induced positive inotropic action (P < 0.02) (Fig. 1).

### Table 3. Subcellular distribution of ³H-ouabain in guinea pig heart

<table>
<thead>
<tr>
<th></th>
<th>Ouabain</th>
<th>Furosemide + Ouabain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Debris</td>
<td>0.18 ± 0.02 (3)</td>
<td>0.27 ± 0.03 (3)</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>0.25 ± 0.01 (4)</td>
<td>0.25 ± 0.03 (3)</td>
</tr>
<tr>
<td>Microsomal fraction</td>
<td>0.77 ± 0.09* (5)</td>
<td>0.57 ± 0.01* (3)</td>
</tr>
<tr>
<td>Supernate</td>
<td>0.10 ± 0.01 (4)</td>
<td>0.07 ± 0.01 (3)</td>
</tr>
</tbody>
</table>

The results indicate the mean ± SEM. Figures in parenthesis refer to the number of animals studied. * Significantly different (P < 0.05) from debris and mitochondria.

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**Fig. 1.** Change in inotropic effect of ouabain in guinea pig papillary muscle. Results indicate mean ± SEM. Figures in parenthesis refer to the number of animal studied. * Significant difference from ouabain (P < 0.02).
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Automaticity induced by Ouabain in the isolated Guinea Pig Papillary Muscle

Fig. 2. A case of automaticity induced by ouabain in guinea pig papillary muscle. Concentration of ouabain: g/ml

Although the isolated papillary muscle of guinea pigs does not contract without electrical stimulation, higher doses of ouabain produced automaticity (Fig. 2). From the results obtained, it was shown that automaticity induced by a dose of $5 \times 10^{-7}$ g/ml was observed in 3 out of 25 cases (12%). On the other hand, after pretreatment with $10^{-6}$ g/ml furosemide, the same concentration of ouabain produced automaticity at a rate of 33.3%. However, this difference was not statistically significant (Table 4).

Table 4. Effect of furosemide on the ouabain-induced automaticity in guinea pig papillary muscle

<table>
<thead>
<tr>
<th>Occurence of automaticity</th>
<th>Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouabain $5 \times 10^{-7}$ g/ml</td>
<td>3/25 (12%)</td>
</tr>
<tr>
<td>Furosemide $10^{-6}$ g/ml + Ouabain $5 \times 10^{-7}$ g/ml</td>
<td>3/9 (33.3%)</td>
</tr>
</tbody>
</table>

The results were expressed in terms of the number of induction/all cases.

DISCUSSION

Little is known about the basic mechanisms of the interaction between ouabain and furosemide (Seller et al., 1975a, 1975b). The most interesting observation in the present experiment was furosemide's potentiation of the toxicity and positive inotropic effect of ouabain in guinea pig papillary muscle. The present investigation was designed to study the proposed mechanism of drug interaction between ouabain and furosemide. Attention was focused on the following two points: the changes in toxic and lethal doses induced by concurrent treatment with ouabain and furosemide; and the influence of furosemide on the binding of $^{3}$H-ouabain by cardiac muscle and by subcellular fractions.

It was shown that furosemide potentiated arrhythmic responses to ouabain both in vivo and in vitro. The potentiation of ouabain-induced arrhythmias after pretreatment with furosemide was antagonized by treatment with potassium sparing diuretic,
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prorenoate (Hofmann et al., 1975). After pretreatment with furosemide, ouabain produced marked arrhythmias without showing any significant changes in its binding of \(^{3}H\)-ouabain by cardiac muscle and by subcellular fractions in guinea pigs. Several investigators have suggested an etiologic relation between the loss of myocardial potassium induced by digitalis and the development of digitalis arrhythmias (Glynn and Karlish, 1975; Gelbart et al., 1980). It is possible that ouabain and furosemide block active cation transport across the cell membrane. And that decreased intracellular potassium induced by dual treatment of ouabain and furosemide might be associated with increased arrhythmogenic effect of ouabain potentiated by furosemide.

As shown in in Fig. 1, the potentiation by furosemide of positive inotropic effect of a subtoxic dose of ouabain was demonstrated in guinea pig papillary muscle. This potentiation may be attributed to the inhibition of transport ATPase activity induced by ouabain and furosemide. To clarify the correlation of cardiac Na\(^{+}\), K\(^{+}\)-ATPase activity with ouabain-induced inotropic stimulation, we measured ouabain sensitive Na\(^{+}\), K\(^{+}\)-ATPase in guinea pig heart. However, good recovery of this enzyme activity was not obtained in guinea pig heart. Indeed, no reports are available in the literature concerning the determination of this enzyme in guinea pig heart. Recent information (Goddard and Robinson, 1975) revealing a new aspect of digitalis action on sodium-calcium exchange mechanism across the membrane points to the proposed possibility that increased intracellular sodium induced by combined treatment of ouabain and furosemide is associated with the increased calcium influx across the cell membrane. The increased intracellular calcium appears to be responsible for the potentiation by furosemide of ouabain-induced positive inotropic action.

**SUMMARY**

Arrhythmogenic responses to ouabain as well as the lowering of its lethal dose were potentiated by pretreatment with furosemide in guinea pigs. Furosemide-induced potentiations of contractile force and arrhythmogenic effect of ouabain were also observed in isolated guinea pig papillary muscle. It was demonstrated that furosemide produced an increase in ouabain toxicity and in positive inotropic effect of ouabain in guinea pigs.

**REFERENCES**


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