THE EFFECT OF FLUOXETINE ON OUABAIN-INDUCED ARRHYTHMIA IN ISOLATED GUINEA-PIG ATRIA

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ABSTRACT
Objective: To study the effect of fluoxetine (Fl), a selective serotonin reuptake inhibitor (SSRI) on ouabain-induced arrhythmia in isolated guinea-pig atria.

Methods: The guinea-pig atrium was dissected out and suspended in modified Krebs solution under physiologic conditions. Drug was added into the solution. The changes in rate and force of contractions were measured using a physiograph. The frozen tissue was suspended in concentrated nitric acid and the ionic content in the resulting solution was measured with a flame atomic absorption spectrophotometer.

Results: Fl (2-16 µg/ml) caused a dose-dependent decrease in rate and contractile force of the isolated guinea-pig atria (p<0.05). Ouabain (1.2 µg/ml) per se produced arrhythmia at 1.5 min and either asystole or standstill at 16 min. Pretreatment with Fl (4 µg/ml) increased the time required to produce arrhythmia by ouabain to 5 min, prolonged the beating of atria to more than 40 min and delayed the occurrence of asystolia. The pattern of contractile force induced by Fl + ouabain was more regular than that produced by ouabain alone. This action of Fl was associated with a reduction of the ionic changes induced by ouabain.

Conclusion: Fl may have a cardiac effect directly to reduce the membrane conductance through ion channels which may decrease ouabain toxicity.

KEYWORDS Isolated atria SSRI ouabain toxicity

INTRODUCTION
It has been reported that tricyclic antidepressant drugs (TCA) such as imipramine and amitriptyline have quinidine-like effect on both atrial and ventricular arrhythmia in therapeutic concentrations. It has also been demonstrated that newly developed antidepressant drugs, selective serotonin reuptake inhibitors (SSRI) show some cardiac effects, though they were specifically developed as non-cardiotoxic agents.

In cardiac tissues isolated from animal hearts, fluoxetine and citalopram were shown to inhibit cardiac Na+ and Ca2+ channels. These direct cardiac electrophysiological effects were found to be similar to those observed for TCA.

The present study was designed to evaluate the influence of fluoxetine (Fl) a SSRI on ouabain-induced arrhythmia in isolated guinea-pig atria. The action of Fl upon net ionic changes by ouabain was also studied.

MATERIALS AND METHODS
Guinea-pigs of either sex weighing between 450-600 gm were anesthetized by ether and exsanguinated. The heart was rapidly removed, the atrium was dissected out in oxygenated modified Krebs solution and suspended in isometric conditions under a tension of approximately 0.5 gm. The temperature of the solution was 36-37°C and the pH 7.4.
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Figure 1. Log dose response curve for the effect of fluoxetine (2-16 µg/ml) on the rate and contractile force of isolated guinea-pig atria after being treated for 20 min.

Figure 2. The effect of ouabain (1.2 µg/ml) alone and ouabain + fluoxetine (4 µg/ml) on contractile force of isolated guinea-pig atria.

After mounting, the preparation was allowed to stabilize for 30 min for equilibration. Rate and force of spontaneous contractions were recorded isometrically with a photosensitive transducer on Beckman RS Dynograph recorder. Ten atria were used for each experiment. Solutions of drugs were prepared so that a constant volume of 0.5 ml for each dose was added to 50 ml of the bathing fluid.

The composition of the modified Krebs solution was as follows (mM): NaCl 118.0, KCl 4.7, CaCl₂ 2.6, MgCl₂ 1.2, NaH₂PO₄ 1.0, NaHCO₃ 25.0, Glucose 11.1, EDTA 0.004 and Ascorbic acid 0.11.

Drugs: Ouabain (Sigma) and fluoxetine (Dista) were used.

Experimental plan

Group I: Twenty atria were examined after a period of 30 min. Fl was added to the bath in doses of 2, 4, 8, 16 µg/ml. To each atrium only one dose of Fl was added.

Group II: Ten atria were treated with ouabain (1.2 µg/ml) alone after a period of 30 min.

Group III: Ten atria were pretreated with 4 µg/ml of Fl for 10 min. Ouabain (1.2 µg/ml) was then added to the bath in the presence of Fl.

Group IV: Ten atria as controls were suspended in the organ bath at the same time as other experiments.

Determination of sodium, potassium and calcium

At the end of the experimental period, each atrium was removed from the bath, blotted between two layers of filter paper and weighed. After being washed out with the sucrose / histidine solution, dissected and frozen atrium was pulverized with a mortar and pestle. The frozen tissue powder was resuspended in concentrated nitric acid and maintained at 60°C for 48 h to allow the tissue to be digested. The resulting solution was diluted with deionized water, and the ionic content was measured with a flame atomic absorption spectrophotometer (Shimadzu 680 AA, Japan).

Statistical analysis: Results were expressed as mean ±SE. Statistical significance was determined by using Student's 't' test for paired data, analysis of variance and Newman-Keul's to compare the ionic changes in four groups. P values <0.05 were considered significant.

RESULTS

Fluoxetine (2-16 µg/ml) caused a decrease in rate (23.1±2.4%- 63.04±11.1%) and force of contraction
Figure 3a. The pattern of contractions of isolated guinea-pig atria at different stages of ouabain (1.2 µg/ml) toxicity.

Table 1. Effect of fluoxetine (4 µg/ml) and ouabain (1.2 µg/ml) on the sodium, potassium and calcium content (µg/gm wet weight, mean±SD) of guinea-pig atria at the end of 20 min.

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>81.65 ± 7.8</td>
<td>157.18 ± 24.4</td>
<td>92.95 ± 18.5</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>79.38 ± 9.0</td>
<td>181.86 ± 24.4*</td>
<td>89.49 ± 12.5</td>
</tr>
<tr>
<td>Ouabain</td>
<td>109.02 ± 20.9**</td>
<td>143.00 ± 32.8</td>
<td>106.43 ± 11.7</td>
</tr>
<tr>
<td>Fluoxetine+ Ouabain</td>
<td>88.86 ± 10.2</td>
<td>156.24 ± 26.7</td>
<td>93.52 ± 15.9</td>
</tr>
</tbody>
</table>

One-way ANOVA

<table>
<thead>
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<th></th>
<th>F</th>
<th>df</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Na⁺</td>
<td>62</td>
<td>23</td>
<td>8.828</td>
</tr>
<tr>
<td>K⁺</td>
<td>62</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Ca⁺</td>
<td>62</td>
<td>23</td>
<td>23</td>
</tr>
</tbody>
</table>

P < 0.05 < 0.001 = 0.07

*P<0.01; **P<0.001 significantly different from respective control. For each experiment n = 10.

Figure 3b. Effect of ouabain (1.2 µg/ml) in the presence of fluoxetine (4 µg/ml) on the isolated guinea-pig atria.

(44.6±4.2% - 66.3±4.4%) of isolated guinea-pig atria in a dose-dependent manner (Figure 1). The action of ouabain (1.2 µg/ml) on the contractile force is given in Figure 2 and Figure 3a. Progressive increase in inotropism of atria was observed a few seconds after ouabain addition. The systolic contractions became irregular (arrhythmia) after 1.5 min (onset of arrhythmia). After 5 min the amplitude decreased, arrhythmia became severe and finally asystolia occurred at the end of 16 min. When ouabain (1.2 µg/ml) was added 10 min after the addition of Fl (4 µg /ml), the onset of arrhythmia significantly increased to 5 min (p<0.005) (Figure 3b). The maximum systolic tension developed by treated atria with ouabain alone was 154.2±5.3% whereas for the preparation treated by Fl + ouabain, it was 163.9±8.4% (Figure 2). Furthermore, the asystolia did not occur
even after 40 min (p<0.05), and the pattern of contractile force by Fl + ouabain was more regular than ouabain alone (Figure 3b).

The ionic content of the treated atria

Tissue content of Na⁺, K⁺ and Ca²⁺ was measured and their values are reported in Table 1. Ouabain (1.2 µg/ml) induced a significant increase in Na⁺ content, but not K⁺ and Ca²⁺ levels (Table 1). Fl alone significantly increased the level of K⁺. There were no differences in Na⁺, K⁺ and Ca²⁺ contents between control and ouabain + Fl groups.

**DISCUSSION**

This study has proved that Fl decreases the rate and contractile force of the isolated guinea-pig atria. These effects are similar to those of fluvoxamine, a SSRI antidepressant which decreased the heart rate and force of contraction in guinea-pig atria³ and the myocardial contractility in rabbit⁷. Our findings indicate that the K⁺ concentration of the atrial tissue is increased by Fl. This action probably may in part explain the negative inotropic and chronotropic action of Fl by hyperpolarization⁸. Fluoxetine shifted a higher membrane permeability of Na⁺ (induced by ouabain), to a lesser extent, whereas increased the intracellular K⁺. Thus the effect of Fl on membrane permeability resembles that of quinidine which stabilizes membrane and prevents Na⁺ influx⁴. Some SSRI, have been demonstrated to show quinidine like effects³. This study indicates that Fl is effective in preventing or delaying ouabain-induced arrhythmia in isolated guinea-pig atria by depressing the sodium and calcium channels⁴ and increasing the atrial tissue level of K⁺ resulting in negative chronotropic and inotropic effects. So our results suggest that cardiac effects of Fl which prevent ouabain-toxicity (arrhythmia) may be due to a quinidine-like action on membrane permeability to correct theionic alterations induced by ouabain.

**REFERENCES**


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