Effect of Amiodarone on Rat Heart Myosin Isoenzymes

Nandalal Bagchi, Thomas R. Brown, David S. Schneider, and Surath K. Banerjee

The effects of amiodarone on heart weight, production of $^{14}$C-CO$_2$ from labelled glucose, myosin ATPase activity, and myosin isoenzyme patterns were determined by comparing control and amiodarone-treated male Wistar rats. Since it has been suggested that amiodarone may interfere with thyroid hormone action on the heart, similar experiments were also carried out in hypothyroid and amiodarone-plus-triiodothyronine (T$_3$)-treated rats, and the data were compared to those obtained in amiodarone-treated rats. Amiodarone treatment for 6 weeks resulted in lower heart weight, decreased atrial production of $^{14}$C-CO$_2$ from labelled glucose, decreased myosin Ca-ATPase activity, and preferential synthesis of $V_3$ isomyosin. These effects were similar to those observed in hypothyroid rats but were lesser in magnitude. T$_3$ treatment of amiodarone-treated rats reversed all the changes induced by amiodarone. Serum thyroxine (T$_4$) was higher in amiodarone-treated than in control rats, while serum T$_3$ was similar. Serum T$_3$ was higher in the amiodarone-plus-T$_3$ than in the amiodarone-treated group. These results show that 1) amiodarone-induced changes resemble hypothyroidism with respect to cardiac myosin expression and atrial CO$_2$ production, 2) amiodarone causes hypothyroid-like changes despite normal serum T$_3$ and increased serum T$_4$, and 3) T$_3$ reverses the effects of amiodarone. These data support the hypothesis that amiodarone inhibits the action of thyroid hormone on the heart. (Circulation Research 1987;60:621–625)

Amiodarone is a potent agent for the treatment of cardiac disorders such as angina and arrhythmias. The drug is used extensively in many countries and has recently been approved for use in the United States. Several studies have suggested that chronic treatment may have effects on the heart that resemble those of hypothyroidism. For example, amiodarone causes prolongation of repolarization in all cardiac tissues, an effect produced by thyroidec- tomy in rabbits. Singh and Vaughan-Williams showed that simultaneous administration of thyroxine (T$_4$) prevented amiodarone-induced lengthening of repolarization of atrial and ventricular action potentials. Amiodarone has been reported to cause bradycardia, prolonged systolic time intervals, and a decrease in Ca$^{2+}$-ATPase activity of cardiac myosin, which are characteristic features of hypothyroidism. The bradycardia is reversed by administration of supraphysiological amounts of triiodothyronine (T$_3$). However, despite findings that suggest hypothyroidism, serum T$_3$, though decreased, remains in the normal range during treatment with amiodarone.

In this report, we have tested the hypothesis that amiodarone may antagonize the effects of thyroid hormone on the heart by examining certain features that are known to be affected by hypothyroidism, e.g., heart weight, rate of metabolism of glucose by the heart, cardiac myosin ATPase activity, and the distribution of myosin isoenzymes. In addition, the effect of exogenous thyroid hormone on amiodarone-induced changes have been examined. The results indicate that the effects of amiodarone mimic the hypothyroid state and that the drug may inhibit the action of thyroid hormone on the heart.

Materials and Methods

Materials

Amiodarone was a gift of Dr. A. Urdang, Sanofi, New York, N.Y. Rat thyrotropin (TSH) was measured using reagents supplied by the National Institutes of Arthritis, Metabolism, and Digestive Diseases.

Animals

Six-week-old normal and thyroidectomized male Wistar rats were purchased from Charles River Breeding Co., Wilmington, Mass. All animals were maintained on Purina rat chow in the form of a fine powder. The various agents were added to the food at concentrations found to deliver the desired daily dose based on estimated food intake of 10 g/rat/day. Water was provided ad libitum.

The animals were divided into several experimental groups and maintained for 3, 6, or 10 weeks. The

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hypothyroid (thyroidectomized) group was given 1 mCi $^{131}$I i.p. and maintained on 1.0% Ca gluconate in the drinking water for the first 2 weeks of the study period. The amiodarone group was given 45 mg/kg body wt/day. The amiodarone-plus-T₃ group received amiodarone for the entire experimental period in addition to 5 μg T₃/kg body wt/day for the final 3 weeks.

**Serum Hormones**

Blood was obtained by cardiac puncture at the time of death. Serum T₄, T₃, and TSH concentrations were determined by standard radioimmunoassay procedures.

**CO₂ Production From Labelled Glucose**

Production of CO₂ from labelled glucose by atrial tissue was measured following the procedure described by Sheer and Morkin. Briefly, freshly isolated atria were washed with cold calcium-free Krebs bicarbonate buffer (pH 7.4) and then incubated in 0.5 ml of the same buffer supplemented with 7 μCi of d-[U-$^{14}$C] glucose for 2 hours at 37°C with gentle shaking. The reaction mixture was gassed with 95% O₂ and 5% CO₂, and the evolved $^{14}$CO₂ was trapped in scintillation vials containing 3 ml Carbosorb (Packard, Downers Grove, Ill.). The vials were replaced every 30 minutes. Ten milliliters of Permifluor 5 (Packard) were added to each vial prior to counting.

**Preparation of Myosin**

Myosins from left ventricular tissues of each experimental group were prepared simultaneously according to the methods described previously. Myosin was extracted from minced ventricular tissue for 15 minutes at 4°C by Guba/Straub’s buffer (pH 6.8) containing EDTA 1 mM, diithiothreitol (DTT) 1 mM, and Na₃P₂O₇ 5 mM. The suspension was centrifuged at 10,000×g for 10 minutes, and the crude myosin was precipitated by tenfold dilution of the supernatant with ice-cold denaturated water containing DTT 1 mM and EDTA 1 mM. The precipitate was dissolved in Tris-maleate 0.05 M (pH 7.5), MgATP 10 mM, DTT 1 mM, and KCl 0.6 M and centrifuged for 90 minutes at 130,000×g to remove traces of actin. Saturated (NH₄)₂SO₄ was added to the supernatant and the myosin, which precipitated at 35–45% saturation, was collected by low speed centrifugation. The purity of the myosin preparations was confirmed by sodium dodecyl sulfate (SDS) gel electrophoresis, which showed virtual absence of actin and other contaminating proteins. The optical density 280/260 ratios for all myosins were within 1.40–1.70.

For native isomyosin analysis, crude myosin was prepared by the method of Hoh et al or by dissolving the crude myosin precipitate described above into a buffer that contained Na₃P₂O₇ 0.1 M, pH 8.8, DTT 10 mM, and glycerol 50%.

**Isomyosin Analysis and ATPase Assays**

Native isomyosins were analyzed by electrophoresing myosins under non-denaturing conditions in pyrophosphate polyacrylamide gel at 3°C for 20 hours using a running buffer, sodium pyrophosphate 20 mM (pH 8.8), cysteine 2 mM, and glycerol 10%. Staining, destaining, and densitometric scanning of the gels were then performed for estimation of the various isomyosins. These techniques were essentially the same as those of Hoh et al, and have been described previously.

The Ca²⁺-ATPase and K⁺(EDTA)-ATPase activities of myosin were measured in duplicate at 30°C in 0.05 M Tris-HCl buffer (pH 7.5), containing 0.5 M KCl by the methods described previously. Concentration of Ca²⁺ and ATP were 10 mM and 5 mM, respectively. For K⁺(EDTA)-ATPase measurements Ca²⁺ was omitted and 5 mM EDTA was included.

**Analytical Techniques**

Myosin concentration was determined by using an absorbance of E₂₈₀ 1% = 0.56. SDS gel electrophoresis was carried out according to Weber and Osborn. Statistical differences between mean values were determined by Student’s t test. Probability values smaller than 0.05 were considered significant. Densitometric scanning of the isomyosin bands in the gel were performed by using Helena Cliniscan Densitometer (Helena Laboratories, Beaumont, Tex.) for processing.

**Results**

**Growth and Serum Hormones**

Table 1 shows serum hormones and gross anatomic data of rat hearts after 6 weeks of treatment. Body weight and heart weight were decreased in hypothyroid rats after 6 weeks. Administration of amiodarone also caused significant, though less marked, reductions of body weight and heart weight. Serum concentrations of amiodarone and its metabolite, desethyl-

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**Table 1. Effect of Amiodarone on Body Weight, Heart Weight, and Serum Hormones**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Body weight (g)</th>
<th>Heart weight (mg)</th>
<th>Ventricle weight $\times 10^3$</th>
<th>Serum $T_4$ (ng/dl)</th>
<th>Serum $T_3$ (μg/kg)</th>
<th>Serum TSH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>369.6 ± 6.9</td>
<td>1,164 ± 83</td>
<td>3.00 ± 0.18</td>
<td>4.8 ± 0.3</td>
<td>82 ± 3</td>
<td>208 ± 45</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>153.5 ± 5.5</td>
<td>406 ± 23</td>
<td>2.48 ± 0.14†</td>
<td>&lt;0.1*</td>
<td>31 ± 2*</td>
<td>8,834 ± 610*</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>353.5 ± 9.2†</td>
<td>930 ± 18†</td>
<td>2.62 ± 0.06†</td>
<td>8.8 ± 0.8*</td>
<td>83 ± 3</td>
<td>239 ± 31</td>
</tr>
<tr>
<td>Amiodarone + $T_3$</td>
<td>346.5 ± 9.0</td>
<td>1,032 ± 53</td>
<td>2.84 ± 0.15</td>
<td>2.5 ± 0.3*</td>
<td>97 ± 4†</td>
<td>71 ± 12*</td>
</tr>
</tbody>
</table>

Six-week-old rats were divided into 4 experimental groups, as described in the text, and killed 6 weeks later. Values are mean ± SEM of groups of 6–8 rats.

*p<0.001, †p<0.01 vs. control.

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amiodarone, were 0.5 ± 0.04 mg/l and 0.1 ± 0.01 mg/l, respectively (determinations were performed in the laboratory of Professor P. Somani, Medical College of Ohio, Toledo, Ohio). Treatment of amiodarone rats with T₃ for 3 weeks increased body and heart weights to the normal range.

Serum T₄ concentration was increased in the amiodarone group while serum T₃ was unchanged, resulting in an increased T₄/T₃ ratio. Serum TSH was not altered. As expected, the hypothyroid group had markedly elevated serum TSH and very low concentration of serum T₄ and T₃. The animals given T₃ with amiodarone had elevated T₃ and decreased T₄ and TSH concentrations, indicating suppression of the pituitary-thyroid axis.

**Atrial CO₂ Production**

Values for ¹⁴C₀₂ production from labelled glucose are shown in Figure 1. CO₂ production was decreased in both hypothyroid and amiodarone-treated groups. Although exogenous T₃ significantly increased labelled CO₂ production in the amiodarone group, the values remained below those of controls.

**Isomyosin Analysis and ATPase Activity of Myosin**

Figure 2 represents a typical pyrophosphate-polyacrylamide gel electrophoresis pattern of ventricular myosin from control, hypothyroid, amiodarone-treated (6 weeks), and amiodarone-plus-T₃-treated rats. As expected, myosin from control and hypothyroid rats showed the predominance of the high ATPase V₁ and the low ATPase V₃ isomyosins, respectively. It is clear that treatment with amiodarone-induced synthesis of both V₂ and V₃ isomyosins, while the myosin from rats treated with amiodarone plus T₃ showed only V₁ isomyosin, indicating that T₃ reversed the effects of amiodarone. The effects of duration of amiodarone treatment on the appearance of V₂ and V₃ isomyosins were also examined. In all, crude myosin from 6 rat hearts from each of these groups were analyzed. Figure 3 shows a representative densitometric scanning pattern.

Amiodarone treatment for 3 and 6 weeks induced preferential synthesis of V₂ isomyosin by 30 ± 2% over control. Treatment with amiodarone for 10 weeks increased the synthesis of V₂ by about 40 ± 3%. It should be noted that, although amiodarone-induced preferential synthesis of V₂ isomyosin, the induction was less than that observed in the hypothyroid group (Figure 1).

Table 2 shows the Ca²⁺- and K⁺(EDTA)-ATPase activities of purified myosin from the various groups. The Ca²⁺-ATPase activities are consistent with isomyosin changes shown in Figure 2. After 6 weeks, ATPase activities were decreased in myosin from the amiodarone-treated group when compared to control (p < 0.02) and returned to normal values in the amiodarone-plus-T₃ group. Assuming that the Ca²⁺-ATPase activity of 0.53 µmol/mg/min found in myosin from hypothyroid rat hearts represents activity solely contributed by V₁ isomyosin, the decrease in ATPase activity by 0.17 units probably corresponds to the presence of only 30% of V₁ in the amiodarone-treated rat heart. The K⁺(EDTA)-ATPase activities of myosin from all groups were the same. Table 2 also shows that treatment with amiodarone for 10 weeks further de-
creased the myosin ATPase by about 11%, which is consistent with the increase in V₃ isoenzyme synthesis described above.

Discussion

The present study shows that treatment with amiodarone decreased heart weight, metabolism of glucose to carbon dioxide by atria, and cardiac myosin ATPase activity with a concomitant increase in the low ATPase V₃ isoenzyme. These changes were similar, albeit to a smaller extent, to those observed in the thyroidec- tomized animals and occurred despite normal serum T₃ and TSH and elevated serum T₄ concentrations. The hormone values are in agreement with a previous report and are most likely due to the inhibition of peripheral 5'-deiodinase rather than the effect of iodine released from the drug. Supraphysiologic concentrations of T₃ reversed all of the amiodarone-induced changes. These data indicate that amiodarone caused hypothyroid-like changes in the heart.

Our observation that amiodarone decreased myosin Ca-ATPase activity is in agreement with an earlier report. The present study shows that this is due to increased accumulation of the low ATPase V₃ isoenzyme. Changes in the isoenzyme pattern and the Ca²⁺-ATPase activity associated with amiodarone treatment are similar to those observed in several experimental cardiac abnormalities, such as pressure overloading and hypothyroidism. Although factors responsible for myosin expression have not been clearly identified, thyroid hormone has been implicated as a modulator of altered thyroid state on atrial intracellular potentials. Am J Physiol 1984;246:R961-968

Table 2. Ca²⁺- and K⁺(EDTA)-ATPase Activities of Myosin From Control and 6 Weeks Amiodarone-Treated Rat Hearts

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Ca²⁺-ATPase (µmol/mg/min)</th>
<th>K⁺(EDTA)-ATPase (µmol/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.20 ± 0.05</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>0.50 ± 0.03*</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Amiodarone-treated 3 weeks</td>
<td>1.06 ± 0.04†</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>6 weeks</td>
<td>1.03 ± 0.04†</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>10 weeks</td>
<td>0.92 ± 0.03†</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Amiodarone + T₃ (5 µg/day)</td>
<td>1.25 ± 0.08</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>

*p<0.001, †p<0.02 vs. control.

The inhibition of thyroid hormone action may explain some of the cardiac effects of amiodarone. Decreased substrate metabolism, as well as reduced contractility resulting from decreased myosin ATPase activity, may reduce the oxygen demand on cardiac muscle. This in turn may explain the antianginal properties of the drug. Indeed, therapeutic induction of hypothyroidism has been used in the past for the treatment of intractable angina. Whether the antithyroid effects also account for the antiarrhythmic properties of amiodarone is not known.

The mechanism by which amiodarone may inhibit the cardiac effect of thyroid hormone remains to be determined. The drug may block intracellular generation of T₃ from T₄ by its well-known inhibitory effect on 5'-deiodinase. The contribution of this pathway to nuclear T₃ content in the heart is not known and may be important. The elevation of serum T₃, found in the amiodarone-plus-T₃ group, may be enough to compensate for the loss of this contribution. On the other hand, ipodate, which also blocks 5'-deiodinase in the heart, does not produce bradycardia or have antiarrhythmic effects. Alternatively, amiodarone could block any of the many steps mediating the tissue effects of T₃, e.g., transport across cell or nuclear membranes, cytosolic binding, nuclear receptor binding, or postreceptor effects. Amiodarone may prove to be an important tool in the elucidation of the cellular processes that mediate the action of thyroid hormone.

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