

## Long-term Effects of Amiodarone and its Non-iodinated Analogue, Dronedarone, on the Transcription of Cardiac Sarcoplasmic Reticulum Ca<sup>2+</sup>-ATPase Gene

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**Abstract:** Antiarrhythmic effects of long-term amiodarone is supposed to be mediated in part by cardio-selective hypothyroidism, but the issue remains controversial. We examined mRNA expression of sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA2) by Northern blot analysis in rats treated with amiodarone (60mg/kg/day, 4 weeks) or dronedarone (60mg/kg/day, 4 weeks). SERCA2 mRNA level in ventricular muscle was significantly decreased in hypothyroid rats by 38±9% ( $p<0.05$ , n=4), whereas increased in hyperthyroid rats by 39±19% ( $p<0.05$ , n=4) compared with control (euthyroid) rats. Amiodarone treatment caused a slight decrease in SERCA2 mRNA (by 20±13%, n=4), whereas dronedarone treatment caused a slight increase (by 19±23%, n=3), although the changes did not reach statistical significance. Amiodarone and dronedarone have different effects on the gene transcription of SERCA2 in the heart, suggesting their different thyroid hormone-mediated actions.

**Key words:** amiodarone, dronedarone, thyroid hormone, SERCA2

### Introduction

Amiodarone is considered the most promising antiarrhythmic agent for the treatment of life-threatening arrhythmias in patients with structural heart diseases (CAST Investigators, 1989; Waldo et al, 1996; Cairns et al, 1997; Julian et al, 1997). The potent antiarrhythmic action of long-term amiodarone is supposed to be mediated at least in part by cardio-selective hypothyroidism, although the issue remains controversial (Kodama et al, 1997).

Thyroid hormones are known to affect twitch contraction of cardiac muscle through altered expression of Ca<sup>2+</sup> handling proteins including sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA), ryanodine receptor and sarcolemmal Na/Ca exchanger. In the hyperthyroid heart, expression of SERCA and RyR is increased, whereas expression of phospholamban (PLB) and Na/Ca exchanger is decreased (Carr and Kranias, 2002). In the hypothyroid hearts, expression of SERCA and RyR is decreased, whereas expression of PLB and Na/Ca exchanger is increased (Carr and Kranias, 2002).

In the present study, we investigated the long-term effects of amiodarone and its non-iodinated benzofuran analogue, dronedarone, on the SERCA mRNA in the left ventricle of rat hearts in comparison with those of systemic hyperthyroidism and hypothyroidism.

### Materials and Methods

The experiments were performed according to protocols approved by the Animal Experimentation Committee, Research Institute of Environmental Medicine, Nagoya University.

#### Animal Treatment

Four-week-old male Wistar rats were assigned to 5 groups: 1) control, 2) hypothyroidism, 3) hyperthyroidism, 4) amiodarone treatment, and 5) dronedarone treatment. Each group contained 3 to 4 rats. Rats of systemic hypothyroidism were prepared by addition of 0.025% methimazole (2-mercapto-1-methyl-imidazole, MMI; Sigma Chemical Co., St. Louis, MO, U.S.A) to the drinking water for 4 weeks. Systemic hyperthyroidism was induced by a daily intraperitoneal injection of 10 µg triiodothyronine (T<sub>3</sub>)/100g body weight. For amiodarone and dronedarone treatment groups, the drugs were administered to rats orally for 4 weeks (60 mg/kg amiodarone or dronedarone daily). Untreated (euthyroid) rats were used as controls. (Takeuchi et al, 2002; Van Opstal et al, 2001; Varro et al, 2001; Sun et al, 1999)

#### RNA extraction and Northern blot analysis

Total RNA was prepared by the acid phenol method as described by Chomczynski and Sacchi. (Chomczynski and Sacchi, 1987) One hundred mg of left ventricular tissue was homogenized with polytron and added to 2 ml solution D (lysis buffer). Solution D consists of 4 M guanidinium thiocyanate

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and 0.2 M 2-mercaptoethanol. Twenty  $\mu\text{g}$  of total RNA per lane was used for Northern blot analysis. After denaturation, samples were separated by 0.8% agarose gel electrophoresis and transferred onto GeneScreen Plus as described previously. The cDNA used as probes for SERCA2 [prepared by RT-PCR using 5'-acgatctgtctctgtgaatgactct-3' (sense) and 5'-ggcgcgtcgttcacaccatcaccagtca-3' (antisense) primers and mouse heart RNA as templates] was synthesized by RT-PCR.(7) The cDNA probes were labeled with [ $\alpha$ - $^{32}\text{P}$ ]dCTP using a random primed DNA labeling kit. Conditions of hybridization and subsequent washing were previously reported. 10) Membranes were hybridized with the labeled probe in a buffer at 42°C for 20 hours. Membranes were then washed twice with 2 $\times$ SSC under the following conditions: 5 min at 20°C, 10 min at 65°C, and 5 min at 20°C. The radioactivity of bands was measured using the Molecular Imager System (BioRad, USA). The accuracy of RNA delivery in Northern Blots was monitored by rehybridization with  $^{32}\text{P}$ -labeled cDNA for 18S ribosomal RNA (rRNA).

Statistical Values Analysis are presented as means  $\pm$  SE unless otherwise specified. ANOVA was employed for statistical analysis. Difference was considered significant at  $p < 0.05$ .

## Results and Discussion

### Animal characteristics

The rats with systemic hypothyroidism showed marked decrease of body weight, whereas those of hyperthyroidism had body weight comparable to control rats (Control vs. Hypothyroid:  $231 \pm 6.0$  vs.  $177 \pm 2.5$  g,  $n=3-4$ ,  $p < 0.05$ ; Control vs. Hyperthyroid:  $231 \pm 6.0$  vs.  $220 \pm 4.1$  g,  $n=3-4$ , NS). Treatment with amiodarone or dronedarone caused no significant changes in the body weight (Control vs. Amiodarone:  $231 \pm 6.0$  vs.  $233 \pm 7.8$  g,  $n=3-4$ , NS; Control vs. Dronedarone:  $231 \pm 6.0$  vs.  $260 \pm 5.8$  g,  $n=3-4$ , NS).

### Northern blot analysis

Figure 1A shows that a representative gel picture of Northern blot for SERCA2 mRNA in the ventricular tissue samples. The bands for 18S rRNA were presented as a reference. SERCA2 mRNA amounts were estimated by densitometry and normalized to the value of 18S rRNA. The data obtained are summarized in Fig. 1B.

Systemic hypothyroidism resulted in a significant decrease of SERCA2 mRNA (by  $38 \pm 9\%$  from control,  $p < 0.05$ ,  $n=4$ ), whereas systemic hyperthyroidism caused a significant increase (by  $39 \pm 19\%$  from control,  $p < 0.05$ ,  $n=4$ ). Amiodarone treatment tended to cause a reduction of SERCA2 mRNA (by  $2 \pm 13\%$  from control,  $n=4$ ) and dronedarone treatment tended to increase the level (by  $19 \pm 23\%$ ,  $n=3$ ), but the changes did not reach a statistical significance. The present data for amiodarone seem concordant with the hypothesis that a long-

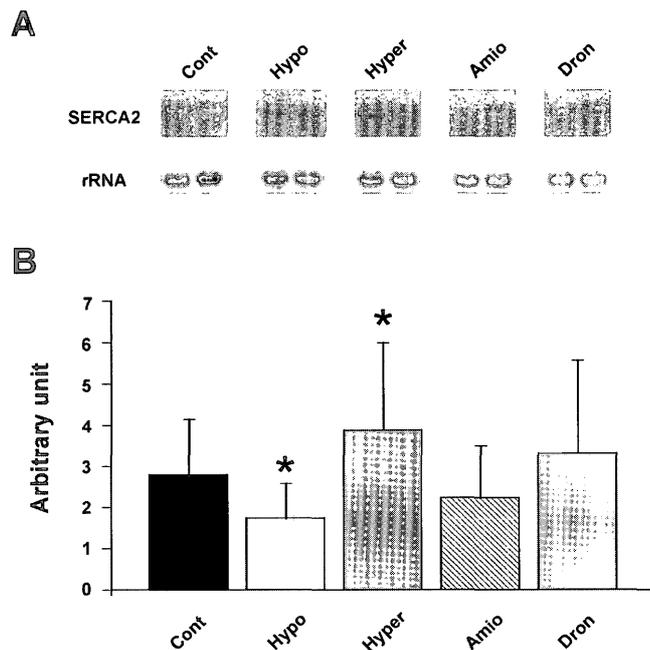


Fig. 1 A. Representative gel pictures of Northern blot analysis for sarco-plasmic reticulum  $\text{Ca}^{2+}$ -ATPase (SERCA). Upper panels show the bands of SERCA in non-treated control, hypothyroid, hyperthyroid, amiodarone-treated and dronedarone-treated rat hearts. Lower panels show the bands of 18S ribosomal RNA used as a reference. B. Summarized data of Northern blot analysis for SERCA gene. The radioactivity of SERCA bands was measured using the Molecular Imager System and normalized with the radioactivity of 18S rRNA bands. Data are presented mean  $\pm$  SE. \* $p < 0.05$  compared to control.

term treatment with this drug causes a cardioselective hypothyroidism. More extensive molecular biological studies on mRNA and protein in the hearts will be required to substantiate the hypothesis.

Despite of potent antiarrhythmic effects, amiodarone has a wide range of extracardiac side effects (Harris et al, 1983), which has been attributed, at least in part, to the iodine in its chemical structure and can significantly diminish its use in clinical practice. Therefore, a non-iodinated benzofuran analogue of amiodarone, dronedarone (SR 33589) was developed to diminish the side-effects. In the present study, dronedarone showed different effects on SERCA gene expression compared to amiodarone, indicating a fundamental difference in molecular targets of these two drugs used for long periods.

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