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Short communication

Beneficial effects of amiodarone in heart failure: interaction with β-adrenoceptors rather than G proteins

Petra Schnabel *, Florian Mies, Christoph Maack, Stephan Rosenkranz, Oliver Zolk, Michael Böhm

Klinik III für Innere Medizin der Universität zu Köln, Joseph-Stelzmann-Str. 9 50924, Cologne, Germany

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Abstract

The effect of the antiarrhythmic drug amiodarone on the human myocardial β-adrenoceptor-G protein-adenylyl cyclase signalling cascade was investigated. Amiodarone had no effect on myocardial G proteins and maximal adenylyl cyclase activity, but acted as a β-adrenoceptor antagonist. This mechanism might be at least partially responsible for the beneficial effects of the drug in patients with arrhythmia and heart failure.

Keywords: Heart failure; Amiodarone; β-adrenoceptor; G protein; Adenylyl cyclase

1. Introduction

Amiodarone is a class III antiarrhythmic drug of increasing importance in patients with heart failure and severe ventricular arrhythmia. A risk reduction of the occurrence of arrhythmic deaths in patients with heart failure of ischemic or non-ischemic etiology has been shown in a number of clinical trials such as BASIS, CAMIAT, EMIAT, GESICA and CHF-STAT (Singh, 1997 for review). In addition, left ventricular function was improved in patients treated with amiodarone (Singh et al., 1995; Massie et al., 1996) indicating mechanisms of action beyond its antiarrhythmic properties. However, these are not yet thoroughly elucidated on the molecular level. Inhibition of one or more K^+ outward currents and the resulting prolongation of action potential and effective refractory period (Freedman and Somberg, 1991; Kowey et al., 1997 for review) have been demonstrated. In addition, antagonism between thyroid hormone and amiodarone was observed (Yin et al., 1994; Drvota et al., 1995a,b). Amiodarone was also reported to compete with ouabain for specific binding sites on myocardial Na^+K^+-ATPase (Almotrefi et al., 1997). The improvement of left ventricular function was also speculated to be due to the inhibition of the production of tumor necrosis factor-α (TNF-α) in human mononuclear cells by amiodarone (Matsumori et al., 1997). Moreover, the drug exerts anti-adrenergic effects. Like β-adrenoceptor antagonists, amiodarone increases left ventricular ejection fraction in patients with heart failure (Cleland et al., 1996 for review), a disease which is characterized by increased sympathetic tone. Besides β-adrenoceptors, G_i proteins are a possible target of amiodarone. Hagelüken et al. (1995) observed a direct activation of pertussis toxin-sensitive G proteins in HL-60 human leukemia cells. However, this mechanism has not yet been investigated in human myocardium.

2. Materials and methods

2.1. Myocardial tissue

Human myocardial tissue was obtained during cardiac transplantation of patients with terminal heart failure due to dilated cardiomyopathy. Explanted hearts were trans-

* Corresponding author. Tel.: +49-221-478-6207; Fax: +49-221-478-6550

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ported from the operation room to the laboratory within 5 min and snap-frozen in liquid nitrogen.

2.2. Membrane preparation

The membrane preparations for radioligand binding studies and for the measurement of adenylyl cyclase activity were performed as described elsewhere (Zolk et al., 1998).

2.3. Radioligand binding studies

Binding studies of β-adrenoceptors were performed as described (Zolk et al., 1998) using [^{125}I]-cyanopindolol as radioligand.

2.4. Adenylyl cyclase activity

Adenylyl cyclase activity was assessed as described (Zolk et al., 1998) using [^{32}P]α ATP as a substrate.

2.5. Isolation of rat cardiomyocytes and measurement of single cell shortening

Cardiomyocytes from adult Sprague–Dawley rats were isolated and single cell shortening of these cells was measured as described elsewhere (Rosenkranz et al., 1997).

3. Results

The effect of amiodarone on the β-adrenoceptor-G protein-adenylyl cyclase signal transduction cascade in membrane preparations from human left ventricular myocardium was investigated.

Adenylyl cyclase activity in human myocardial membranes was increased about 1.5-fold by the β-adrenoceptor agonist isoproterenol at 100 μM (Fig. 1A). This response was blunted and the curve was shifted to the right when the incubation was performed in the presence of amiodarone. In contrast, the G protein activator Gpp(NH)p stimulated adenylyl cyclase activity to a similar extent in the absence and presence of amiodarone (Fig. 1B). Maximal adenylyl cyclase activity as assessed by stimulation with forskolin (Fig. 1C) was also similar in the absence and presence of amiodarone. These results suggest an antagonistic action of amiodarone at β-adrenoceptors, while they provide evidence against interactions of the drug at the G protein level or the catalytic subunit of adenylyl cyclase. In addition, [^{32}P]ADP-ribosylation of human myocardial G_i proteins was not affected by overnight pre-incubation with amiodarone (not shown).

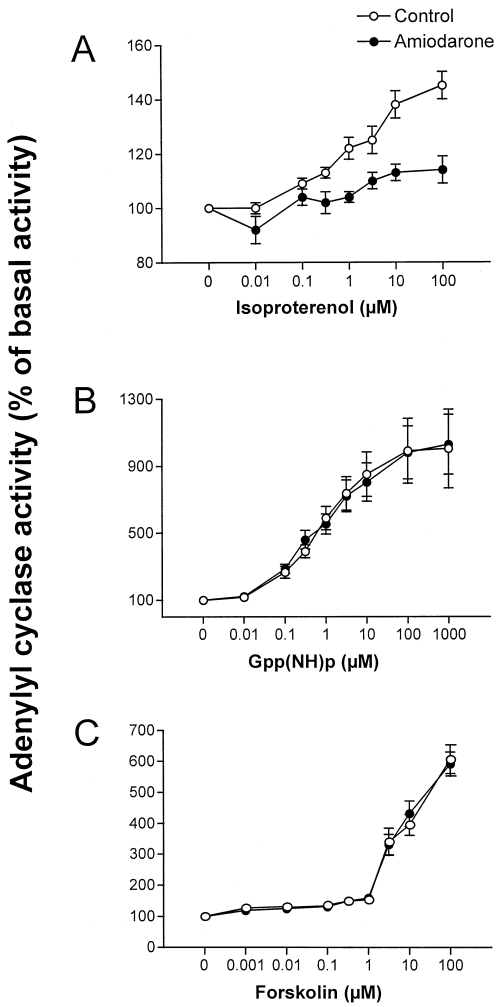

Fig. 1. Effect of amiodarone on myocardial adenylyl cyclase activity. Membrane preparations from human left ventricular myocardium (25 μg protein/tube) were prepared and adenylyl cyclase activity was determined in the absence (open circles) and presence (closed circles) of amiodarone (100 μM). Panel A, concentration dependence on isoproterenol. Panel B, concentration dependence on Gpp(NH)p. Panel C, concentration dependence on forskolin. Data shown are means ± S.E.M. of six left ventricular samples from six explanted human hearts.

In order to characterize the antagonistic effect of amiodarone at β-adrenergic receptors, radioligand binding experiments were performed in which amiodarone displaced the nonselective radioligand [^{125}I]-cyanopindolol (Fig. 2A). The IC_{50} value for amiodarone was 29 μM. β-adrenoceptor subtype selectivity of amiodarone was investigated by displacement experiments in the presence of β_1- and β_2-selective antagonists and revealed a slight, 1.8-fold, selectivity for β_2-adrenoceptors (not shown).

The effect of amiodarone on the myocardial contractile response to β-adrenergic stimulation was investigated by measuring the shortening of isolated rat cardiomyocytes. Fig. 2B shows the concentration dependence of the β-adrenoceptor agonist isoproterenol after overnight pre-incubation with vehicle or amiodarone, respectively. Pre-incubation with amiodarone (Fig. 2B) shifted the curve to

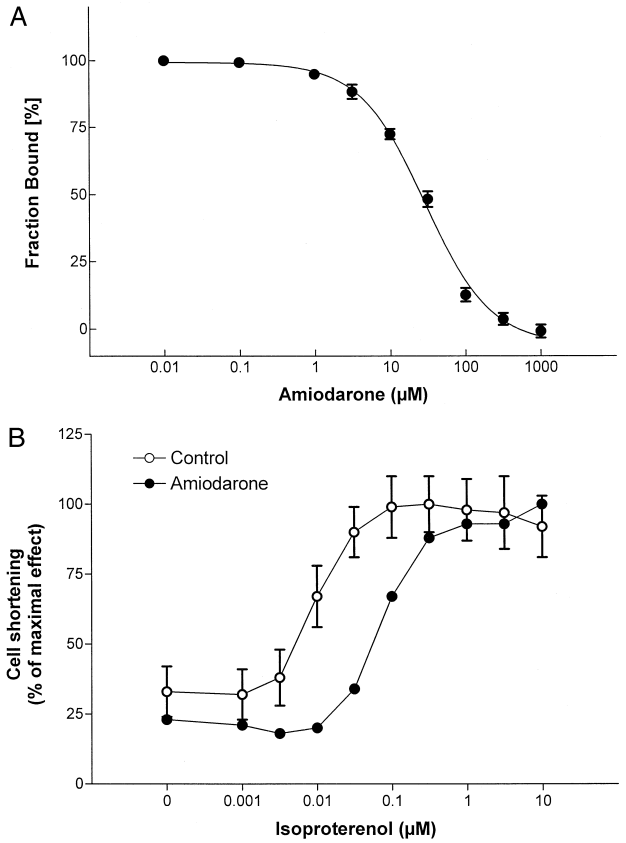

Fig. 2. (A) Binding of amiodarone to myocardial β-adrenoceptors. Membrane preparations from human left ventricular myocardium (25 μg protein/tube) were prepared and specific binding of the radioligand [^{125}I]-cyanopindolol was displaced by increasing concentrations of amiodarone. Data shown are means ± S.E.M. of three left ventricular samples from three explanted human hearts. (B) Effect of amiodarone on cardiomyocyte shortening. Adult rat cardiomyocytes were isolated and isoproterenol-dependent shortening of single cells was assessed after 12 h of incubation in medium without amiodarone (open circles, $n = 12$) and medium containing amiodarone (10 μM; closed circles, $n = 4$), respectively.

the right by one order of potency. These results provide further evidence for a β-adrenoceptor-antagonistic effect of amiodarone.

4. Discussion

The assessment of adenylyl cyclase activity suggests an antagonistic action of amiodarone at β-adrenoceptors, while the experiments provide evidence against interactions of the drug at the G protein level or the catalytic subunit of adenylyl cyclase. These findings were supported by the observation that [^{32}P]ADP-ribosylation of human myocardial G_i proteins was not affected by overnight pre-incubation with amiodarone.

Radioligand binding experiments in which amiodarone displaced the nonselective β-adrenoceptor antagonist [^{125}I]-cyanopindolol revealed an IC_{50} of the drug lying in the micromolar range. This indicates a rather low affinity of amiodarone for β-adrenoceptors. However, serum levels (Gonska, 1993; Kowey et al., 1997) have been shown to lie in the micromolar range, too, indicating that the β-adrenoceptor-antagonistic properties of amiodarone might be relevant in vivo. Moreover, Candinas et al. (1998) showed that myocardial tissue levels of amiodarone and its metabolite desethylamiodarone correlate with the cumulative ingested dose of the drug and thus, can amount to even higher concentrations after long-term treatment. This study also demonstrated that the tissue concentrations of the lipophilic drug vary greatly between different regions of the heart. Highest drug levels were obtained in epicardial fat tissue (Candinas et al., 1998). These findings indicate that myocardial contractility in response to amiodarone should not be measured in isolated papillary muscle strips from explanted human hearts, as the degree of fibrosis and the content of adipose tissue is difficult to control. Therefore, the effect of amiodarone on the myocardial contractile response to β-adrenergic stimulation was investigated by measuring the shortening of isolated rat cardiomyocytes. The rightward shift of the concentration–response curve of isoproterenol is also consistent with a β-adrenoceptor-antagonistic effect of amiodarone. β-adrenoceptor antagonists are known to exert antiarrhythmic effects as well as to improve ejection fraction in patients with heart failure (Cleland et al., 1996, for review). In contrast, our results argue against a direct effect of amiodarone on myocardial G proteins. Additional mechanisms such as the reduction of cytokine production might also be involved in the beneficial effects of amiodarone on left ventricular function in heart failure and deserve further examination.

In summary, amiodarone exerts β-adrenoceptor-antagonistic effects in human myocardium which might contribute to the antiarrhythmic properties of amiodarone, as well as to the increase of ejection fraction in heart failure.

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