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Interaction of propranolol with S100 proteins of the cardiac muscle

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Abstract

The cardioprotective activity of propranolol is believed to be independent of its β-adrenoceptor antagonistic effect. Propranolol exerts this effect through a direct effect on the cardiac muscle, but the precise mechanism remains unclear. In this work, we demonstrated that propranolol binds to S100ao and S100L proteins with ED_{50} of approximately 1.0 μM without cation dependency and that this binding changes the conformation of these S100 proteins. Propranolol, however, was found to bind to and to change the conformation of S100C protein in the presence of Mg^{2+} or Zn^{2+} with ED_{50} of approximately 1.0 μM. No change was observed in the presence of Ca^{2+}. Moreover, in the presence of Mg^{2+}, the ED_{50} of L- and D- propranolol were approximately 0.8 and 2.0 μM, respectively. This study demonstrated for the first time, that the S100 proteins of the cardiac muscle are intracellular targets of propranolol, and that Mg^{2+} is a modulator of the cardioprotective activity of S100C protein.

Keywords: β-Adrenoceptor antagonist; Propranolol; S100 protein; Cardiac muscle; Mg^{2+}

1. Introduction

β-Adrenoceptor antagonists are widely used for the treatment of a variety of cardiovascular disorders such as hypertension, angina pectoris and cardiac arrhythmias. They improve the cardiac contractile performance and myocardial relaxation leading to an overall increase in the efficiency of the cardiac work. β-Adrenoceptor antagonists probably exert their effect by hemodynamic improvement and sympathetic stimulation (Held and Yusuf, 1993). However, β-adrenoceptor antagonists are generally contra-indicated in patients with heart failure. Recently, long-term oral therapy with β-adrenoceptor antagonists has been shown to improve symptomatology in patients with chronic heart failure (Kelly, 1993). It was also reported that β-adrenoceptor antagonists improve survival in patients with myocardial infarction, even in the presence of heart failure (Doughty et al., 1994). The hypothesis that β-adrenoceptor antagonists might be important for the treatment of heart failure is now being widely accepted. However, the mechanism of this cardioprotective activity is unclear. For the more effective indication of β-adrenoceptor antagonist for the treatment of heart failure, it is important to clarify their mechanism of action. In the present study, we assessed the mechanism of action of propranolol which is one of the most extensively studied and widely used β-adrenoceptor antagonist.

The diverse cardioprotective mechanisms of propranolol depend on different pathways that lead to β-adrenoceptor antagonism. The cardioprotective effects of propranolol that produce functional improvements has been previously investigated using whole heart or isolated muscles. Regarding the intracellular action of propranolol, it was previously found that it interacts with Ca^{2+}-binding proteins such as calmodulin (Katz et al., 1974; Volpi et al., 1981), troponin C (Su and Malencik, 1985), and protein kinase C (Sozzani et al., 1992). However, the dissociation constants (K_d) between propranolol and these proteins are much higher than those needed to antagonize β-adrenoceptors (Boucher et al., 1992), and than those needed to produce cardioprotection. Thus, propranolol seems to interact with different proteins for exerting its cardioprotective activity.

To identify other molecular targets of propranolol, precipitates of heart homogenate were applied to propranolol-Sepharose 6B. Various factors were found to bind to propranolol-Sepharose 6B in a Ca^{2+}-dependent fashion. Two major bands corresponding to a molecular mass of about 17 and 10 kDa were observed. The band with a molecular mass of 17 kDa probably corresponds to calmodulin (Katz et al., 1974; Volpi et al., 1981). The other band (10 kDa) corresponded to S100 proteins as

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judged on their tissue distribution, molecular mass and the Ca^{2+} dependency of their binding to propranolol-Sepharose 6B. The interaction of S100 proteins with propranolol has not been as yet reported. In this paper, we purified S100ao, S100L and S100C proteins from cardiac muscle which is known to contain proteins predominantly of the S100 protein family, and studied their interaction with propranolol, using affinity column chromatography with propranolol-Sepharose 6B and fluorescence analysis.

2. Materials and methods

2.1. Purification of proteins

S100C protein was purified from porcine heart following a previously reported procedure (Naka et al., 1994). S100ao and S100L proteins were purified from bovine heart as described previously (Donato et al., 1989; Glenney et al., 1989). Concentration of proteins was determined by Bio-Rad protein assay reagent using bovine serum albumin as standard (Bio-Rad).

2.2. Affinity chromatography

Immobilized propranolol columns were prepared using propranolol and epoxy-activated Sepharose 6B according to the manufacturer's instructions (Pharmacia). Purified proteins (S100C, S100ao and S100L) were applied to propranolol-Sepharose 6B column (volume: 1 ml) in the binding buffer (50 mM Tris-HCl, pH 7.4, 1 mM $CaCl_2$ or 1 mM $MgCl_2$ or 1 mM $ZnSO_4$) and eluted with the same buffer containing 3 mM EGTA or EDTA instead of cations. The S100C protein in the eluted fractions (1 ml each fraction) was monitored at absorbance 259 nm, and S100ao, S100L proteins at 280 nm. Protein fractions were then analyzed by SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue R-250.

2.3. Fluorescence analysis

In order to examine the change in the conformation of S100 proteins, purified S100ao, S100L and S100C proteins were labeled with the fluorescent probe, 5-dimethylamino-1-naphthalenesulfonyl chloride (dansyl chloride; Sigma). Fluorescence of dansylated S100 proteins were monitored by a spectrofluorometer (RF-5000; Shimadzu, Kyoto, Japan). Excitation was adjusted to 335 nm, and fluorescence was obtained at 455 ± 3 nm. The relative fluorescence change was expressed as the percentage of each maximal change in fluorescence of dansylated S100 proteins after subtracting the background light intensity measured with buffer alone. In experiments using stereoisomers of propranolol, changes in fluorescence were expressed in relation to the initial fluorescence of each dansylated S100C protein in the absence of propranolol. For each experiment, three to four replicate assays were performed and the data expressed as the mean $\pm$ standard error (S.E.) of the mean. The difference between the mean values were analyzed by ANOVA. P values of less than 0.01 were considered as statistically significant.

3. Results

3.1. Binding of S100 proteins to propranolol-Sepharose 6B

Each S100 protein was applied to column chromatography with propranolol-Sepharose 6B in the presence of 1 mM $CaCl_2$. S100ao and S100L proteins bound to the column in the presence of $CaCl_2$, but they did not elute with buffer containing EGTA. Even in the absence of $CaCl_2$, both proteins bound to the column. In contrast, S100C protein passed through the column in the presence or absence of $CaCl_2$ (Fig. 1, fractions 1–10).

In the presence of 1 mM $ZnSO_4$ or 1 mM $MgCl_2$, S100ao and S100L proteins were bound to the propranolol-Sepharose 6B column but did not elute with EDTA-containing buffer. However, in the presence of 1 mM $ZnSO_4$ or 1 mM $MgCl_2$, S100C protein bound to the column and eluted with EDTA-containing buffer (Fig. 1, fractions 11–20). S100C protein did not bind to the column in the absence of 1 mM $ZnSO_4$ or 1 mM $MgCl_2$.

3.2. Fluorescence analysis

Conformation change of dansylated S100 proteins was analyzed by fluorescence study.

In the presence of 1 mM $MgCl_2$, propranolol produced significant changes in the fluorescence of dansylated S100 proteins, the ED_{50} being approximately 1.0 μM (Fig. 2A). Maximal enhancement was observed at propranolol concentration of more than 10 μM. The difference between changes in fluorescence intensity induced by S100 proteins was not statistically significant. In the presence of 1 mM

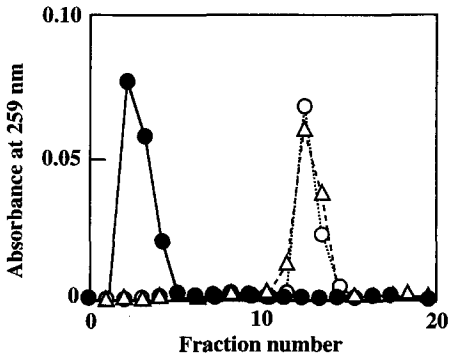

Fig. 1. Cation-dependent affinity chromatography of S100C protein using propranolol-Sepharose 6B column. Absorbance of eluted fractions was determined at 259 nm. The column was first washed with equilibrating buffer containing 1 mM $CaCl_2$ (●), 1 mM $MgCl_2$ (○), 1 mM $ZnSO_4$ (△) (fraction 1–10) and then elution of proteins was performed with buffer containing 3 mM EGTA/EDTA (fraction 11–20).

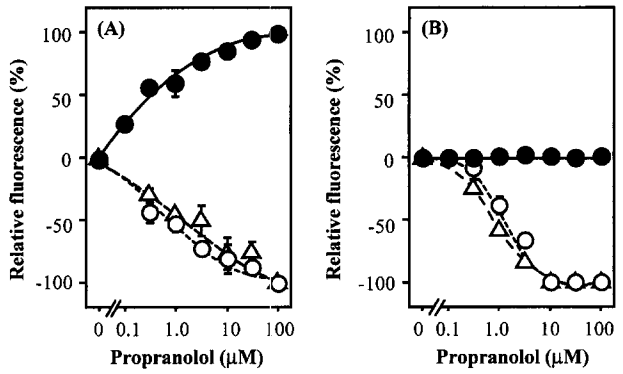

Fig. 2. Effects of propranolol on the fluorescence of dansylated S100C (●), S100ao protein (○) and S100L proteins (△) (50 mM Tris-HCl, pH 7.4) under various conditions; (A) in the presence of 1 mM $MgCl_2$; (B) in the presence of 1 mM $CaCl_2$. λex = 335 nm: λem = 455 ± 3 nm. Data shown are the means ± S.E. of three experiments.

$ZnSO_4$, the titration curves and the half maximal changes in fluorescence of all dansylated S100 proteins were similar to those observed in the presence of $MgCl_2$.

In the presence of $CaCl_2$, propranolol induced similar changes in the fluorescence of dansylated S100ao and dansylated S100L proteins, but they were not statistically significant. In contrast, propranolol had no effect on the fluorescence of dansylated S100C protein (Fig. 2B).

3.3. Stereoisomers of propranolol

As shown in Fig. 3, both two optical isomers of propranolol induced changes in the fluorescence of dansylated S100C protein in the presence of $MgCl_2$. These changes were dose-dependent, the ED_{50} being 0.8 and 2.0 μM for L- and D-propranolol, respectively. The magnitude of the fluorescence change induced by L-propranolol was about twice of that induced by D-propranolol and it was statistical significance ($P < 0.01$) at concentrations from 0.1 μM to 3 μM.

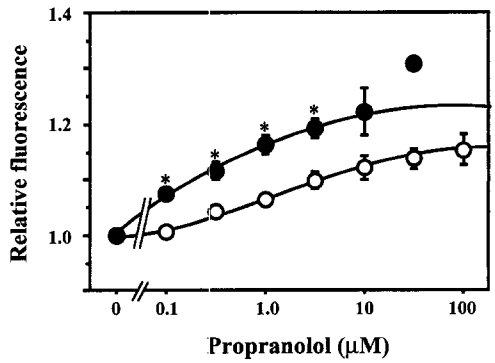

Fig. 3. Comparative evaluation of the effect of L-propranolol (●) and D-propranolol (○) on the fluorescence of dansylated S100C protein in the presence of 1 mM $MgCl_2$. λex = 335 nm: λem = 455 ± 3 nm. Data shown are the means ± S.E. of four experiments. * $P < 0.01$ vs. the same concentration of D-propranolol.

4. Discussion

In this study, S100 proteins were found to bind to propranolol. Cation-dependent chromatography performed with propranolol-Sepharose 6B column revealed that the interaction between S100C protein and propranolol depends on Mg^{2+} and Zn^{2+}, whereas the interactions of S100ao and S100L were Mg^{2+}- and Zn^{2+}-independent. The fluorescence analysis also disclosed the same cation dependency. The concentration of propranolol required to bind to each S100 protein was similar, the ED_{50} being about 1.0 μM. This is the concentration at which propranolol exerts a cardioprotective effect (Yue et al., 1992), and it is lower than that required to interact with other Ca^{2+}-binding proteins, such as calmodulin (Katz et al., 1974) or troponin C (Sozzani et al., 1992). It was suggested that S100 proteins are intracellular target molecules of propranolol and probably exert other actions besides their β-adrenoceptor antagonistic activity. To analyze the mechanism of regulation of this effect, the influence of some cations was evaluated.

In the presence of Mg^{2+}, the binding of Mg^{2+} to S100C protein and to S100ao and S100L proteins showed opposite effects in the fluorescence analysis, namely, the quantum yield increased for S100C but decreased for S100ao and S100L. The effect of Mg^{2+} on the structure of S100C, S100ao and S100L showed differences in the behavior of these proteins.

Previous reports showed that Ca^{2+} and Zn^{2+} bound to S100ao and S100L proteins, and induced changes in the conformation of these proteins, whereas Mg^{2+} was found to be ineffective (Baudier and Gerard, 1983; Glenney et al., 1989). Regarding the biological function of S100A1 protein, the influence of Ca^{2+} and Zn^{2+} on the regulation of giant protein kinases by this protein was recently reported (Heierhorst et al., 1996). In this work, the binding of these cations did not affect the interaction of S100ao and S100L with propranolol; that is, the conformation change of these proteins after binding to Ca^{2+} or Zn^{2+} did not modify the propranolol-binding sites on these S100 proteins. S100C protein was also sensitive to both Ca^{2+} (Naka et al., 1994) and Zn^{2+}. However, Ca^{2+} did not affect the propranolol-S100C interaction, whereas Zn^{2+} altered the binding of S100C protein to propranolol. This is the first observation showing that S100C protein conformation is affected by Mg^{2+}. Conformation changes induced by Zn^{2+} and Mg^{2+} might modify the propranolol-binding sites on S100C protein. Of these two cations, Mg^{2+} was found to modulate the interaction of S100C protein with propranolol at physiological concentrations. Since these S100 proteins highly conserved their primary structures, such as the EF-hand type Ca^{2+}-binding domains, it appears that the propranolol-binding site on S100C protein is different from those on S100ao and S100L proteins, and that this S100C protein exhibits a different

propranolol-dependent conformational change as compared to other members of the S100 protein family.

In previous studies, it was reported that L-propranolol preferentially interacts with β-adrenoceptors, and that D-propranolol has less than 1% of the potency of L-propranolol for antagonizing β-adrenoceptors (Barrett and Cullum, 1968; Howe and Shankes, 1966). However, unlike this striking different antagonizing effect against β-adrenoceptors, these two propranolol stereoisomers have an almost similar degree of cardioprotective activity. The conformation change of S100C protein induced by L-propranolol was only twice of that induced by D-propranolol. These data suggested that, besides antagonizing β-adrenoceptors, propranolol binds to S100C proteins and thus exerts cardioprotective activity. S100 proteins have been reported to play also various other intracellular biological functions such as activation of aldolase, inhibition of phosphorylation of τ protein, and inhibition of adenylate cyclase (Baudier and Cole, 1988; Fano et al., 1988; Zimmer and Van Eldik, 1986). Recently, it has also been reported that S100A1 participates in the regulation of protein kinase and that S100 protein induces activation of photoreceptor guanylate cyclase (Heierhorst et al., 1996; Margulis et al., 1996). In this paper, we demonstrated a novel intracellular function of S100 proteins. Further works must be carried out to clarify the other physiological functions of S100 proteins.

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