Sera Antibodies of Patients with Dilated Cardiomyopathy: Arrhythmogenic Potential due to Delay in the Final Repolarization Phase

T. CHRIST, E. WETTWER, H.M. HIMMEL, G. WALLUKAT[1], U. RAVENS
Department of Pharmacology and Toxicology, Dresden University of Technology, Dresden, Germany
[1]Max Delbrück Center for Molecular Medicine, Berlin, Germany

Summary

Antibodies against many myocardial antigens are found in the sera of patients with dilated cardiomyopathy. Some of them can affect cell function by reacting with the ß-adrenoceptor, i.e. increase the beating frequency of cultured rat cardiomyocytes. Therefore, we studied the influence of antibodies on action potentials of adult rat cardiomyocytes. Antibodies were prepared by ammoniumsulfate precipitation and purification by affinity chromatography. Rat ventricular cells were exposed with antibody preparations of three different patients with dilated cardiomyopathy. Action potentials were elicited in current clamp mode (0.5 Hz, 37 °C). AP were analyzed at 90 and 30 % repolarization (APD_{90}; APD_{30}). The antibodies significantly and reversibly increased APD_{90} within 1 - 2 min of exposure but did not alter the resting membrane potential. The antibody-induced prolongation in APD_{90} were: antibody from patient 1: 7.5 ± 1.5 ms (n = 4 cells); patient 2: 2.9 ± 0.9 ms (n = 7); patient 3: 4.5 ± 0.9 ms (n = 12). In comparison, isoprenaline (1 µM) prolonged APD_{90} to a similar extent as the antibodies, but induced a significantly larger increase in APD_{30} than the antibodies from patient 3, i.e. 9.3 ± 3.4 ms vs. 1.2 ± 0.3 ms ($p < 0.04$). Antibodies from sera of patients with DCM produce changes in adult rat cardiomyocytes, that differ from those with isoprenaline. The electrophysiological effects of antibodies may suggest an arrhythmogenic potency of antibodies in patients with DCM.

Key Words

Cardiomyopathy, repolarization, antibodies

Introduction

Dilated cardiomyopathy is defined by progressive dilatation and loss of function of the ventricle in the absence of known cause [1]. It has been claimed that autoimmune responses against various myocardial antigens are involved in its pathogenesis [2]. Anti-peptide antibodies against the ß-receptor and antibodies from sera of patients with DCM react with synthetic peptides derived from the ß-adrenoceptor. They are able to affect ß-receptor-mediated functions in animal cells [3,4]. Autoantibodies against the ß-receptors are found in 10 % of patients with ischemic cardiomyopathy and in 26 % of patients with dilated cardiomyopathy and are associated with poor left ventricular function [5]. Removal of antibodies by immunoadsorption has been shown to improve left ventricular function over a period of months [6].

Sudden cardiac death is a major cause of mortality in patients with DCM and is estimated to be responsible in 50 - 82 % of deaths [7]. Though it may be difficult to distinguish between sudden cardiac death caused by ventricular arrhythmias or exacerbation of pump failure, it is widely accepted that ventricular arrhythmias are a major part of mortality in these patients.

Therefore, the aim of our study was to test antibodies from patients with DCM for possible electrophysiological effects. We chose to explore this question with an analysis of action potentials of rat ventricular cells exposed to antibodies from sera of patients with DCM.

Methods

Patients

The patients whose antibodies (AB) were prepared, had been hospitalized for congestive heart failure and were treated with diuretics, digitalis and angiotensin-converting enzyme inhibitors. Coronary heart disease was excluded based on coronary angiography, and no patient had a history of hypertension, valvular heart disease, diabetes, or any other endocrinal disorder or suspicion of alcohol or drug abuse.

All patients had depressed left ventricular function (ejection fraction) as judged from noninvasive investigation by M-mode and two-dimensional echocardiography.

Preparation of the immunglobulin fraction

The immunglobulin fraction was isolated from 2 ml serum samples by ammonium sulfate precipitation at a saturation of 40 %. The precipitates were washed and dissolved in dialysis buffer (154 mM NaCl, 10 mM sodium phosphate; pH 7.2). Finally, the immunglobulins were taken up in 2 ml phosphate-buffered saline (10 mM phosphate, 150 mM NaCl, pH 7.4). The immunglobulins were then adsorbed in a affinity column that was made up by coupling the peptide 197-222 of the second extracellular loop of the ß$_1$-adrenoceptor to sepharose. The purified ABs were dialyzed against the phosphate-buffered saline. Antibodies were stored at -20 °C until use. Only AB preparations that have a positive chronotropic effect on spontaneously beating cultured nenonatal rat cardiomyocytes were employed.

Cell isolation

Male Wistar rats (body weight 200 - 250 g) were killed by cervical dislocation. As described previously, the hearts were perfused on a Langendorff apparatus at 37 °C for 5 - 7 min with nominally Ca^{2+}-free Tyrode (composition in mM: 150 NaCl, 5.4 KCl, 10 HEPES, 2 $MgCl_2$, 20 glucose, adjusted to pH 7.4 with NaOH) [8]. The rat hearts were then perfused for 15 min with collagenase-containing Tyrode (collagenase Type I Worthington, 233 units/mg; 0.54 mg/ml)) supplemented with albumin (1 mg/ml). After tissue digestion, the hearts were taken off the perfusion apparatus and tissue batches from the apex and the base of the heart were dissected, and their dissociation was continued separately to obtain subendocardial and subepicardial myocytes, respectively [9]. Only subendocardial cells were used for action potential recording. Single ventricular cells were collected in a low Ca^{2+} Tyrode (composition in nM: 100 NaCl, 10 KCl, 1.2 KH_2PO_4, 5 $MgSO_4$, 50 taurine, 5 MOPS and 50 glucose) and the Ca^{2+}-concentration was slowly increased until a final concentration of 0.5 mM was reached. The cells were stored at room temperature and used within 8 h.

Electrophysiological studies

Cardiomyocytes were transferred to a small recording chamber (volume 50 µl), inserted on a heated platform, mounted on an inverted microscope (IMT-2, Olympus). The chamber was continuously perfused at a constant rate (0.8 ml/min) with heated solution. A heated superfusion system was used for application of AB and drugs. By using this system, an AB saving procedure was possible.

Only rod-shaped myocytes with clear striations were used. For action potential recording the single-electrode voltage clamp technique was applied. Heat polished pipettes were pulled from borosilicate filament glass (OD 1.5 mm) and had a tip resistance of 3.5 - 5.0 MΩ. Current clamp was achieved using an Axopatch 200 amplifier (Axon instruments, Forster City). For stimulus protocol design and data acquisition, the ISO 2 software (MFK) was used. All experiments were carried out at 37 °C.

Measurement of action potentials

At the beginning of each experiment, a hyperpolarizing pulse was applied in order to determine the membrane capacitance. Action potentials in rat myocytes were measured in the current clamp mode after injection of current (duration 2 ms, amplitude 0.8 - 1.0 nA, stimulation rate 0.5 Hz). Pipettes were filled with a solution containing (in mM) 40 KCl, 8 NaCl, 100 DL-potassiumaspartat, 5 Mg-ATP, 5 EGTA, 2 $CaCl_2$ 10 HEPES, 0.1 GTP-Tris adjusted to pH 7.4 with KOH. Thus, the free Ca^{2+} concentration was buffered to 50 nM (free Mg^{2+} concentration 700 µM) as calculated by the computer program EQCAL (Biosoft). The bath solution was composed (in mM): 150 NaCl, 5.4 KCl, 10 HEPES, 2 $MgCl_2$, 2 $CaCl_2$, 20 glucose adjusted to pH 7.4 with NaOH.

Action potentials were analyzed for resting membrane potential (RMP) and for action potential duration at 30 and 90 % of repolarization (APD_{30}, APD_{90}). Action potentials were elicited 5 min after establishing the

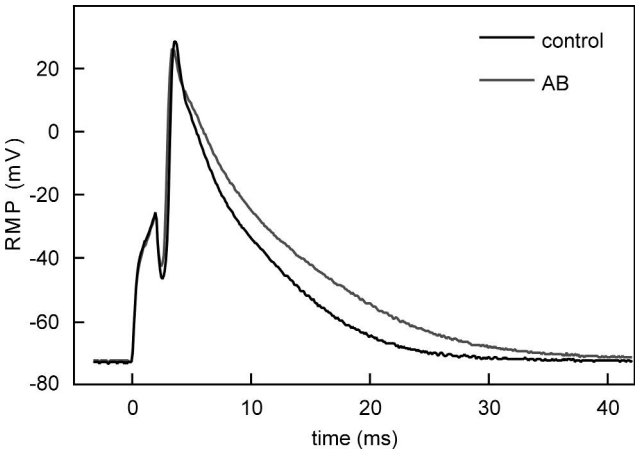

Figure 1. Action potentials of a rat ventricular myocyte in the absence (control) and presence of AB from patient 3 (dilution 1:50). Stimulation rate 0.5 Hz.

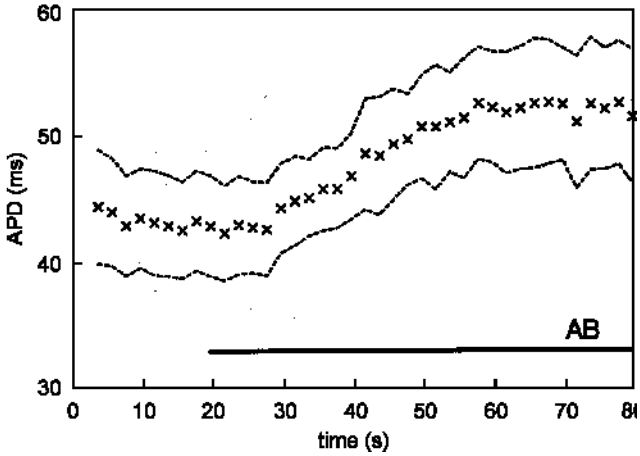

Figure 2. Time course of prolongation of APD_{90} of rat ventricular cells during exposure over 60 sec with AB from patient 1 (dilution 1:50). Mean values ± S.E.M. (n = 4).

whole-cell configuration. To minimize flow and temperature artifacts due to the superfusing system, control AP were recorded only after 1 min superfusion with tyrode solution. The flow through the superfusion system was never stopped, only switched from one to another channel. Cells were stimulated continuously during the experiment.

The effects were measured within 1 - 2 min of exposure of AB or isoprenaline. For any value, five APs were averaged.

Time-matched control experiments were performed with switching the Tyrode flow in the superfusing system in corresponding time intervals.

Chemicals

(-)-Isoprenaline was purchased from Sigma.

Data analysis

Results are presented as mean values ± S.E.M. Parameters were averaged from five action potential (AP). Differences were evaluated statistically by t-test for paired data and considered significant if $p < 0.05$. Analysis of variance (ANOVA) followed by Turkey-test was used for comparison between groups.

Results

Action potential waveforms differ between subendocardial and subepicardial myocytes [9]. To minimize the variance in APD, we used only subendocardial cells for our analysis. Under control condition RMP was -72.8 ± 1.9 mV and APD_{90} 51.8 ± 12.6 ms.

During time-matched control experiments (n = 9), neither RMP or APD changed significantly (data not shown).

The dilution of AB preparations in terms of the serum volume from which it was prepared was 1:50 (v/v) This dilution was chosen because it had a marked positive chronotropic effect on spontaneously beating cultured neonatal rat cardiomyocytes [4]. Exposure to AB significantly increased APD_{90}. A typical example is shown in Figure 1. The onset of the effect was rapid and occurred within 10 sec of AB addition (Figure 2). The increase in APD was reversible and reproducible (Figure 3). RMP was not altered by AB. The magnitude of increase in APD_{90} for any AB preparation is shown in Figure 4. The increase in APD_{90} was considered significant for all three tested AB preparations ($p < 0.05$), and was significantly greater for AB from pat. 1 compared to pat. 2 ($p < 0.05$).

As it was assumed, that AB from the sera of patients with DCM affect cell function by interacting with the ß-adrenoceptor, we performed experiments with (-)-isoprenaline, a typical ß-adrenoceptor agonist for comparative purposes. The concentration of isoprenaline in all experiments was 1 µM to achieve maximal or near maximal stimulation of the ß-adrenoceptor. Isoprenaline prolonged the APD_{90} to a similar extent as

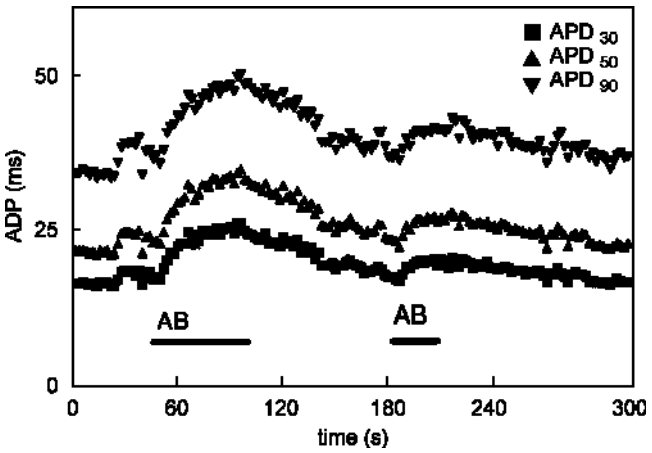

Figure 3. Time courses for the effects of AB on APD_{30}, APD_{50}, and APD_{90} during exposure and re-exposure to AB from patient 2 (dilution 1:50).

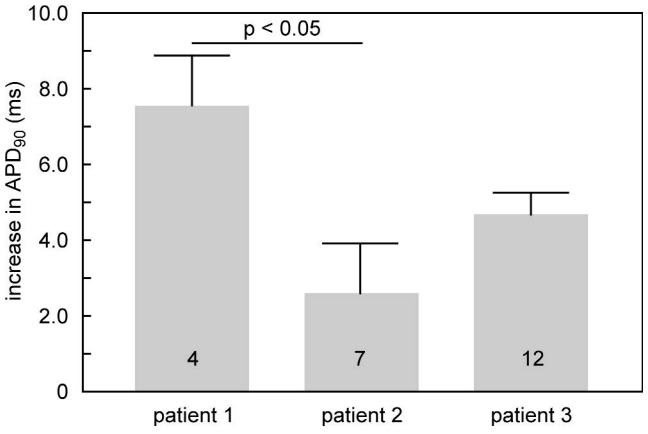

Figure 4. AB-induced increase in APD_{90} in rat ventricular myocytes. Mean values ± S.E.M. calculated from experiments with AB preparations on three different patients. Number of experiments as indicated in the columns.

the AB. But the isoprenaline-induced increase in APD_{30} was significantly greater than the increase obtained with AB, i.e. 9.3 ± 3.4 ms vs. 1.2 ± 0.3 ms (AB from pat. 3).

In Figure 5, the relationship between APD_{30} and APD_{90} during the development of the effects in response to either AB or isoprenaline have been plotted. The myocyte was first exposed to AB from pat. 2 followed a period of wash out (100 sec) and then to isoprenaline (1 µM). The start of either exposure is marked with an arrow. The increase in APD by AB is based mainly on a delay of the final phase of the repolarization whereas the ß-adrenoceptor agonist causes a more uniform prolongation of both APD_{30} and APD_{90}.

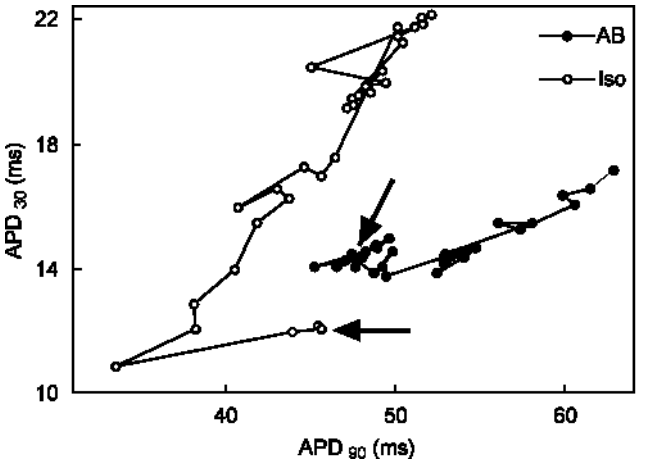

Figure 5. Effect of AB and (-)-isoprenaline (1 µM) on APD_{30} and APD_{90} in rat ventricular myocytes. Cell was exposed first to AB and after wash out from AB (100 sec) to isoprenaline. The start of any exposition is marked with an arrow. Points represented the APD for the single AP (stimulated at 0.5 Hz, distance between two points is 2 sec). Isoprenaline influenced APD_{90} in a biphasic fashion and to a lesser extent as AB. The prolongation of APD_{30} by AB is small compared to the effect of isoprenaline.

Discussion

Recently it was shown that immunglobulins from the subclass IgG can leave the vasculature and accumulate in the myocardium [10]. Therefore, AB in the serum of patients with DCM is expected to be able to reach the heart muscle cell, even in the absence of inflammation of vessels. Hence, AB may play a pathogenic role not only in myocarditis, but also in DCM and ICM, since inflammatory responses are not a prerequisite for AB penetration into the heart.

AB from sera of patients with DCM are able to increase cAMP concentrations in Sf9 insect cells expressing the native human ß-adrenoceptor [5]. The increase in cAMP content was less than with isoprenaline. Similar data were shown for cultured neonatal rat cardiomyocytes [11]. Interestingly, the maximal positive chronotropic effect of the AB on the spontaneous beating frequency of these cells was smaller than that of isoprenaline. Therefore, it has been sug-

gested that AB do not act as typical agonist but rather as activators or sensitizers of the ß-adrenoceptor. In our experiments, AB affected APD in the same direction as isoprenaline (prolongation). In contrast to isoprenaline, AB affected mainly the phase of final repolarization, suggesting a mechanism different from that of pure ß-adrenoceptor stimulation.

The differences in prolongation of APD between AB preparations of patient 1 and 2 are difficult to interpret for the time being. It could reflect different serum concentrations of the AB or different potencies of the AB from the two patients.

Delaying the phase of final repolarization of the action potential may be considered as potential arrhythmogenic like the stimulation of the sympathetic system. AB are present in more than 25 % of patients with DCM and in 10 % in ICM. This high prevalence underline the importance of a potential role for arrhythmias in patients with heart failure. ABs are associated with poor left ventricular function [5], but could also be an additional independent risk factor for ventricular arrhythmias.

Conclusion

The mechanism of how AB affect AP remains to be investigated. The prevalence of such antibodies in a population with ventricular arrhythmias, i.e. patients with ICD, has to be compared to patients with DCM but no arrhythmias, in order to test whether AB could have a role in the risk stratification for arrhythmias. Binding of free AB or inhibiting their binding to the target site at the heart muscle cell are potential options for treatment of patients with arrhythmias in addition to drugs or implantable electrical devices.

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