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Dephosphorylation of Connexin43 Associated with Ventricular Hypertrophy

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Abstract: Altered expression and distribution of gap junctions in hypertrophied hearts may provide a potential substrate for abnormal conduction. We investigated changes of phosphorylation state of connexin43 (Cx43) in hypertrophied rat right ventricles secondary to pulmonary hypertension induced by monocrotaline (MCT) using western blot analysis. In normal right ventricular myocardium, most of Cx43 was phosphorylated. In hypertrophied ventricles of MCT-treated rats, the non-phosphorylated isoform of Cx43 increased and the phosphorylated isoform of Cx43 decreased with no significant a change in the total amount of Cx43 protein. These results suggest that dephosphorylation of Cx43 in MCT-induced right ventricular hypertrophy may be involved in gap junction disorganization.

Key words: gap junction, connexin, cardiac hypertrophy, dephosphorylation, immunoblotting

Gap junctions are specialized membrane regions consisting of groups of channels that directly connect the cytoplasmic components of adjacent cells and enable intercellular communication with respect to the exchange of ions and small (< 1 kDa) molecules. Gap junctions are composed of transmembrane proteins that belong to the connexin family. The principal gap junctional protein expressed in the ventricles of mammalian heart is connexin 43(Cx43), which is confined to the intercalated disks at the cell termini.[1)]

Remodeling of gap junction distribution is an important feature of the structural substrates of arrhythmias under various pathological cardiac conditions including ischemia, myocardial infarction and hypertrophy.[2,3)] In a recent immunohistochemical study on rats with ventricular hypertrophy induced by pressure overload, we have shown a marked disorganization of Cx43 in hypertrophied ventricles; Cx43 expression was not confined to the intercalated disks at the cell termini but was dispersed widely over the entire sarcolemma.[4,5)]

Increasing evidence has focused attention on the role of phosphorylation in the regulation of connexin stability and degradation. For example, phosphorylation of Cx43 on Ser255 in Rat1 cells promotes endocytosis and degradation of Cx43.[6)] In contrast, it has been suggested that dephosphorylation of cardiac Cx43 induced by ischemia is associated with translocation of Cx43 gap junctions into intracellular pools.[7)] However, it is not known whether changes in Cx43 phosphorylation state is involved in gap junction remodeling associated with ventricular hypertrophy.

In present study, we investigated changes in phosphorylated and non-phosphorylated isoform of Cx43 in hypertrophied ventricular myocardium by immunoblotting using isoform-specific antibodies.

Materials and Methods

1. RV hypertrophy animal model

5-week-old Wistar rats pulmonary hypertension was induced by intraperitoneal injection of monocrotaline (MCT, 60 mg/kg), as described.[4)] Saline (0.5 ml) was injected into rats as a control study. Animals were sacrificed 4 weeks after the injection to evaluate RV hypertrophy by the heart-to-body weight ratio (HW/BW) and the tissue weight ratio of the RV free wall to the LV free wall plus the interventricular septum (RV/[LV + IVS]). We also estimated cell hypertrophy induced by MCT treatment: single cells were enzymatically isolated from both ventricles, the cell dimension was compaired between MCT-treated and control rats.

2. Immunoblot

The amount of Cx43 was evaluated by immunoblotting of RV homogenates prepared 4 weeks after MCT injection. A rabitt polyclonal antibody (Zymed) directed against epitopes in the C terminus of Cx43 was used for immunoblotting. This antibody to detect both the phosphorylated and non-phospho-

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rylated isoforms of Cx43. We also used a mouse monoclonal antibody (Zymed) which selectively to non-phosphorylated Cx43. The specificity of these antibodies has been demonstrated previously.[8] The intensity of Cx43 bands was quantified by densitometry and normalized to actin.

3. Statistical analysis

Data are expressed as mean ± SE and analyzed by unpaired t-test. A value of $p < 0.05$ was considered to be statostically significant.

Results and Discussion

1. RV hypertrophy in MCT-treated rats

The HW/BW ratio and the RV/(LV + IVS) ratio were significantly larger in MCT-treated rats 4 weeks after the injection than controls (Table 1). The cell width of the MCT-treated RV myocytes was also significantly larger than controls, whereas there was no significant difference in the cell length of RV myocytes. The cell dimension of LV myocytes (both cell width and cell length) of MCT-treated rats was comparable to that of LV myocytes of control rats. (Data not shown.)

2. Characterization of Cx43 phosphorylation isoform by immunoblot analysis

Figure 1A shows a representative blot of RV homogenate with the rabbit polyclonal Cx43 antibody. In a blot prepared from control rats, the polyclonal antibody detected a major band arround 42kDa (P2) and two faint bands at lower molecular weight (P1 and NP). In a blot prepared from MCT-treated rats, the P2 band was less abundant and the NP band was more abundant compared with controls. Two bands (P1, P2) at the higher molecular weight, and a band (NP) at the lower molecular weight comprise phosphorylated and non-phosphorylated isoforms.[9] Accordingly, the most of Cx43 in normal ventricular myocardium may be phosphorylated. Figure 1B shows an immunoblot of the same samples using the mouse monoclonal antibody. The monoclonal antibody detected only a faint band corresponding to a non-phosphorylated Cx43 (NP) in controls. The NP band detected by the monoclonal antibody was more intense in MCT-treated rats.

Densitmetric analysis of immunoblots with the rabbit polyclonal antibody revealed that the average amount of NP Cx43 in MCT-treated rats was higher than controls (1.01 vs 0.69, n=2), whereas the average amount of phosphorylated (P1+P2) Cx43 in MCT-treated rats was lower than control rats (0.76 vs 1.12, n=2). There was no significant difference in the total amount of Cx43 between MCT-treated and control rats (1.75 vs 1.81, n=2). The increase in the NP isoform in MCT-treated rats was confirmed by a quantitative measurement of immunoblots with the mouse monoclonal antibody (0.77 vs 0.47, n=2).

Table 1 RV hypertrophy in MCT - treated rats.

		Control (n = 14)	MCT (n = 14)
BW	(g)	286.2 ± 5.6	244.3 ± 4.7*
HW	(g)	1.13 ± 0.03	1.18 ± 0.04
HW/BW	(%)	0.40 ± 0.01	0.49 ± 0.02*
RV/LV+ IVS	(%)	22.6 ± 0.8	34.9 ± 2.8*
		Control (n = 21)	MCT (n = 174)
Isolated RV myocytes			
Cell width	(μm)	30.1 ± 1.8	45.4 ± 2.3*
Cell length	(μm)	108.8 ± 5.8	121.8 ± 5.5

BW: body weight, HW: heart weight, HW/BW: heart weight to body weight ratio, RV/LV+ IVS: tissue weight ratio of RV free wall to LV free wall plus interventricular septum, *: $p < 0.01$

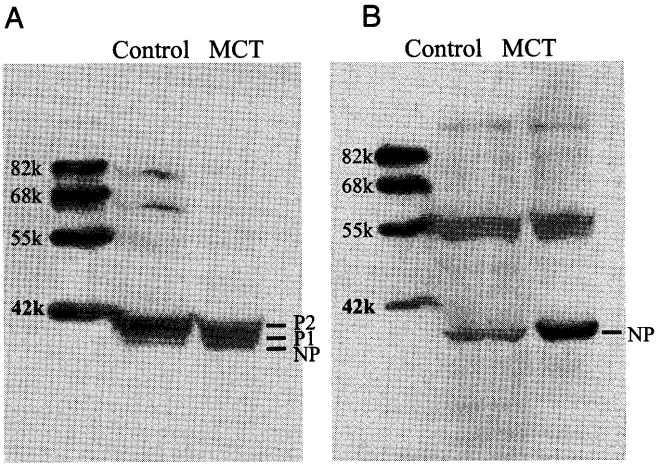

Fig. 1 Immunoblots of RV homogenate of control and MCT-treated rats probed with rabbit polyclonal (A) and mouse monoclonal (B) antibodies for Cx43.

In the present study, we demonstrate that RV hypertrophy secondary to pulmonary hypertension is associated with an increase of NP isoform of Cx43 without a significant change in the total amount of Cx43. In our recent immunohistochemical study using the same RV hypertrophy model, we have shown that marked disorganization of the Cx43 gap junction distribution develops with the progress of RV hypertrophy: Cx43 immunolabeling in hypertrophied RV myocytes was dispersed widely over the entire sarcolemma rather than confined to the intercalated disks at the cell termini.[4] Electron microscopy has shown that Cx43 gap junction remodeling in hypertrophied ventricles includes formation of annular profile of the gap junction membrane suggesting an increased internalization of the junction structure.[10] These observations suggest that the gap junction disorganization in hypertrophied ventricular myocytes is associated with dephosphorylation of Cx43, and translocation of Cx43 from sarcolemmal gap junctions into intracellular pools. In order to substantiate this possibility, morphological studies using immunohistochemistry and

immunogold labelling with isoform-specific Cx43 antibodies will be needed.

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