Phosphorylation of connexin in functional regulation of the cardiac gap junction

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In cardiac muscle, the gap junction contributes to electrical cell-to-cell coupling. This physiological function of the gap junction depends on the phosphorylation state of the connexin molecule, which comprises the gap junction channel. The effects of intracellular Ca^{2+} overload, acidosis, activation of protein kinase (PK) A, PKC and PKG on the phosphorylation and expression of connexin 43 (Cx43) were examined in animal hearts with reference to physiological function. Activation of PKA promotes cell-to-cell coupling due to augmentation of the PKA-mediated phosphorylation of Cx43, with a rise in the quantity of and an increase in the expression of Cx43. A rise in the ionic strength of Ca^{2+} and H^+ impaired cell communication, with the inhibition of PKA-mediated Cx43 phosphorylation. Activation of PKC reduces the quantity and expression of Cx43 despite augmentation of PKC-mediated phosphorylation of the protein. The effects of PKG activation are similar to those of PKC activation. It is suggested that PKA activation upregulates and PKC activation downregulates Cx43. The role of connexin phosphorylation in the regulation of gap junction function is discussed.

Key Words: *Cardiac gap junction; Connexin 43; PKA-mediated phosphorylation; PKC-mediated phosphorylation*

In cardiac muscle, the gap junction greatly contributes to electrical cell-to-cell coupling and intercellular impulse propagation. Therefore, dysfunction of the gap junction is an arrhythmogenic factor because it induces disturbances of conductivity. Electrical coupling of the gap junction is evaluated by gap junction conductance (gj). Generally, gj is described as $gj=N \times Po \times \nu j$, where N, Po and νj represent the number of active channels, open probability of channels and conductance of a single channel, respectively. N, Po and νj are influenced by the phosphorylation of connexin, a phosphoprotein that comprises the gap junction channels.

It is well documented that activation of cyclic AMP-dependent protein kinase (PK) A is the most important factor for increasing gj and promoting electrical cell-to-cell coupling in cardiac cells (1-3). On the other hand, Ca^{2+} (4-9), H^+ (4,6,10,11), activation of PKC (12-14) and activation of PKG (14) decrease gj and degrade electrical cell-to-cell coupling. However, the effects of these factors on the phosphorylation of connexin have not been clarified.

The present review focuses on the effects of the factors influencing gj (activation of PKA, PKC and PKG, and Ca^{2+} and H^+) on the phosphorylation of connexin 43 (Cx43) which is dominantly expressed on ventricular muscle cells. Experiments were performed on hearts isolated from adult guinea pigs and rats. Electrical cell-to-cell coupling in multicellular preparations, namely, junctional resistance, was estimated using longitudinal internal resistance (ri) measured in isolated ventricular muscle strips. Phosphorylation of Cx43 was detected by Western blotting using monoclonal anti-Cx43 antibody. The expression of Cx43 was evaluated by confocal image analysis of immunoreactive micrographs with immunohistochemistry for Cx43.

RESULTS AND DISCUSSION

Effects of PKA activation

In normal conditions, both phosphorylated and unphosphorylated isoforms were detected in immunoblots for Cx43. The phosphorylated isoform of Cx43 was sensitive to and augmented by PKA activators (15).

We have also previously shown that external application of 8-bromocyclic AMP (cyclic AMP) and PKA activators reduced ri in accordance with the augmentation of Cx43 phosphorylation (15). Cyclic AMP and PKA activators increased the quantity of Cx43 protein and the immunoreactive area for Cx43 in the intercalated disks (16-18). These cyclic AMP and PKA activator effects were abolished by PKA inhibitors. PKA-induced phosphorylation of Cx43 is termed PKA-mediated phosphorylation of Cx43. These results suggest that the activation of PKA promotes Cx43 synthesis, accelerates the assembly of connexons into the gap junction plaque and/or inhibits proteolytic degradation of Cx43. These are factors that increase the number of gap junction channels. Our results indicated that activation of PKA increased N and Po.

PKA-mediated phosphorylation upregulates Cx43 and promotes the physiological function of the gap junction, namely, electrical cell-to-cell coupling (16).

Effects of Ca^{2+} and H^+

It has been shown that intracellular Ca^{2+} overload elevated ri in accordance with the inhibition of PKA-mediated

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phosphorylation of Cx43. The Ca^{2+}-induced electrical uncoupling and dephosphorylation were alleviated by acceleration of PKA activation (15). However, the ameliorative effects of PKA were no longer observed when the concentration of Ca^{2+} was markedly elevated (over 1.5 μM) (15). This evidence suggests that PKA-mediated phosphoryation of Cx43 is affected by the ionic strength of Ca^{2+}.

Intracellular acidosis raised ri and suppressed PKA-mediated phosphorylation of Cx43 depending on the extent of the decrease in pH (15). The rise of ri and dephosphorylation were alleviated by acceleration of PKA activation in a pH range of 7.1 to 6.9, but no effect was observed at a pH below 6.5 (15). These results suggest that PKA-mediated phosphorylation of Cx43 is affected by the ionic strength of H^+.

As mentioned previously, it is well documented that Ca^{2+} and H^+ are two important factors that decrease gj; however, it is not clear whether these ions act directly on the gap junction channels and close them. Our results indicate that these ions impair the gap junction function by decreasing Po or νj through dephosphorylation of Cx43.

Effects of hypoxia or ischemia

Disturbances of intercellular impulse conductivity with hypoxia or ischemia may be caused by dysfunction of the gap junction because cell-to-cell coupling has been shown to be impaired during ischemia (19,20). There are reports (21,22) that Cx43 was dephosphorylated during hypoxia and ischemia. Therefore, dysfunction of the gap junction in hypoxia or ischemia may be a result of connexin dephosphorylation. According to previous results from our laboratory (15,23), this dephosphorylation of connexin may reflect an inhibition of PKA-mediated phosphorylation of Cx43 induced by Ca^{2+} or H^+, because both intracellular Ca^{2+} overload and intracellular acidosis occur during hypoxia and ischemia (19,24,25). We have previously reported that an elevation of the intracellular cyclic AMP level prevented and restored hypoxia-induced electrical cell-to-cell uncoupling (26). However, the ameliorative effects of cyclic AMP were not observed when hypoxia continued for more than 1 h (20). The loss of the ameliorative effects of cyclic AMP may be explained by the fact that the ionic strength of Ca^{2+} and H^+ were raised to a level where no ameliorative effect of cyclic AMP was observed (15).

Effects of PKC

It has been reported that activation of PKC decreases gj (12-15) and impairs gap junction communication (25). We observed that PKC activation by 12-O-tetradecanoylphorbol-13-acetate raised ri in accordance with significant augmentation of Cx43 phosphorylation and a decrease in the quantity of Cx43 protein (15-18). We also demonstrated a remarkable reduction in the immunoreactive area of Cx43 in the intercalated disks, rather an increase in the expression on the surface of the membrane (17,18). These effects of PKC were abolished by calphostin C and pretreatment with lysosomal inhibitors (ammonium chloride and leupeptin) (18). These results support the the suggestion that PKC activation inhibits assembly of the connexons into the gap junction plaque and accelerates proteolytic degradation of Cx43 (27,28). This leads to a decrease in the number of gap junction channels in the intercalated disks.

These phenomena of PKC activation have also been observed in tissues other than cardiac cells. In rat liver epithelial cells, degradation of Cx43 due to activation of the proteolysis pathway was observed after Cx43 phosphorylation by PKC (29,30). In mouse embryo, the assembly of Cx43 into the gap junction plaque failed after connexin phosphorylation by PKC (31).Our laboratory has also shown that activation of PKC inhibited PKA-mediated phosphorylation of Cx43 (15-18).

Angiotensin II has been reported to reduce cell-to-cell coupling (32-34). According to our results (15-18), this effect of angiotensin II could be explained by PKC phosphorylation of connexin, because the action of angiotensin II is intracellularly transmitted by PKC activation. It has been reported that the border zones of myocardial infarcts undergo remodelling, which is potentially mediated by angiotensin II (35,36), and increased anisotropy (37,38). Promotion of anisotropy is caused by abnormal distribution and expression of the gap junction (39); in other words, remodelling of the gap junction, which is an arrhythmogenic substrate (40-42). Angiotensin II may induce gap junction remodelling (43,44). Thus, activation of PKC is a potent inducer of gap junction remodelling.

In the streptozotocin-induced diabetic rat heart, it was observed that the conduction velocity of ventricular muscle was reduced in accordance with an increase in ri, the quantity of Cx43 was reduced despite the augmentation of PKC-mediated phosphorylation and immunoreactive expression of Cx43 was reduced in the intercalated disks, particularly on the surface of the membrane (45). These Cx43 expression abnormalities were ameliorated by treatment with lysosomal inhibitors. The alterations in Cx43 were similar to those in 12-O-tetradecanoylphorbol-13-acetate-induced activation of PKC (45). We observed that PKCε was highly activated in the diabetic heart; and it was confirmed by coimmunoprecipitation of Cx43 and PKCε that Cx43 is phosphorylated by PKCε (45). In the diabetic heart, the gap junction may be remodelled by acceleration of PKC activation. This may explain why the diabetic heart is susceptible to arrhythmias.

In summary, Cx43 is highly vulnerable to proteolytic degradation when it is phosphorylated by PKC, and PKC induces the downregulation of connexin and suppression of gap junction function. PKCε may be the isoform of PKC that induces the downregulation of connexin.

Effects of PKG

The effects of PKG activation (by 8-bromocyclic GMP) on electrical cell-to-cell coupling, Cx43 phosphorylation and immunoreactive expression of Cx43 were similar to those of PKC activation (18). It was not possible to conduct analyses using PKG inhibitors. More detailed examinations are required.

CONCLUSIONS

The physiological function of the gap junction is dependent on the phosphorylation of connexins, which comprise the gap junction channels.

PKA-mediated phosphorylation of Cx43 upregulates and maintains Cx43. However, PKC-mediated phosphorylation downregulates and degrades Cx43. These two factors play an important role in the regulation of Cx43 turnover. Acceleration

of PKA-mediated phosphorylation of Cx43 promotes cell-to-cell coupling and conductivity in ventricular muscle cells. However, activation of the proteolytic pathways for Cx43 and suppression of cell communication may occur due to the higher ionic strength of Ca^{2+} or H^{+} that occurs with ischemia, augmentation of PKC-mediated phosphorylation that occurs in the diabetic heart or by excess synthesis of angiotensin II, and augmentation of PKG-mediated phosphorylation, inhibiting PKA-mediated phosphorylation of Cx43. Remodelling of the gap junction due to inhibition of PKA-mediated phosphorylation of Cx43, namely, dephosphorylation of Cx43 or augmentation of PKC-mediated phosphorylation, must be an arrhythmogenic factor (46).

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