Endogenous protein phosphatase 1 runs down gap junctional communication of rat ventricular myocytes

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Endogenous protein phosphatase 1 runs down gap junctional communication of rat ventricular myocytes. Am J Physiol Cell Physiol 281: C1648–C1656, 2001.—Gap junctional channels are essential for normal cardiac impulse propagation. In ventricular myocytes of newborn rats, channel opening requires the presence of ATP to allow protein kinase activities; otherwise, channels are rapidly deactivated by the action of endogenous protein phosphatases (PPs). The lack of influence of Mg2+ and of selective PP2B inhibition is not in favor of the involvements of Mg2+-dependent PP2C and PP2B, respectively, in the loss of channel activity. Okadaic acid (1 μM) and calyculin A (100 nM), both inhibitors of PP1 and PP2A activities, significantly retarded the loss of channel activity. However, a better preservation was obtained in the presence of selective PP1 inhibitors heparin (100 μg/ml) or protein phosphatase inhibitor 2 (12; 100 nM). Conversely, the stimulation of endogenous PP1 activity by p-nitrophenyl phosphate, in the presence of ATP, led to a progressive fading of junctional currents unless I2 was simultaneously added. Together, these results suggest that a basal phosphorylation-dephosphorylation turnover regulates gap junctional communication which is rapidly deactivated by PP1 activity when the phosphorylation pathway is hindered.

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interruption of cell-to-cell communication in ventricular myocytes of newborn rat. Indeed, in these cells, the rapid decline of channel activity in ATP-deprived conditions can be both mimicked by PK inhibition and virtually abolished by nonspecific inhibition of phosphoserine/threonine PPs (31), suggesting that the channel rundown results from dephosphorylation of either the channel-forming protein itself or an associated regulatory protein, induced by endogenous, plausibly membrane-bound PP(s).

Phosphoserine/threonine PPs are classically divided into two classes: type 1 phosphatases (PP1) are inhibited by two heat-stable proteins, termed protein phosphatase inhibitor 1 and inhibitor 2 (I1 and I2), and preferentially dephosphorylate the β-subunit of phosphorylase kinase, whereas PP2 are insensitive to I1 and I2 and preferentially dephosphorylate the α-subunit of phosphorylase kinase (7). PP2 can be further subdivided into PP2A (okadaic sensitive), PP2B (Ca²⁺ dependent), and PP2C (Mg²⁺ dependent).

The aim of the present study was to identify the PP responsible, in rat ventricular myocytes in culture, for the interruption of cell-to-cell coupling when not counterbalanced by PK activities.

METHODS

Cell preparation. Cardiomyocytes were obtained from neonatal (1- to 2-day-old) Wistar rats that were killed by cervical dislocation and then decapitated. Heart ventricles were minced into small pieces (~1 mm³), washed in a Ca²⁺- and Mg²⁺-free medium (Spinner’s solution) that contained (in mM) 116 NaCl, 5.3 KCl, 1.0 CaCl₂, 0.8 MgCl₂, 25 NaHCO₃, 10 glucose (pH 7.4), and incubated in the cell incubator for 15 min to complete ester hydrolysis. After washing, the cells were centrifuged at 500 g for 10 min. The cell pellets were resuspended in Ham’s F-10 culture medium (GIBCO, Cergy-Pontoise, France) supplemented with 10% fetal calf serum (Boehringer), 10% heat-inactivated horse serum (GIBCO), penicillin G (100 IU/ml; Sigma, Saint Quentin Fallavier, France), and streptomycin (50 IU/ml; Sigma). They were preplated in large polystyrene dishes (Nunclon, Roskilde, Denmark) to allow the attachment of nonmuscle cells. The cardiac myocytes in the supernatants were counted, diluted with culture medium to reach a final concentration of 30,000 cells/ml, and seeded (~55,000 cells/cm²) in 35-mm plastic petri dishes (Nunclon). Finally, the cells were incubated at 37°C in a CO₂ incubator (5% CO₂-95% ambient air, giving a pH of 7.4). After 24 h, the culture medium was replaced by a culture medium devoid of fetal calf serum. The experiments were performed after 2 or 3 days of culture; the dishes were transferred onto the stage of an inverted microscope, and the cells were observed by phase-contrast microscopy. The spontaneous synchronized mechanical activity was used as evidence to avoid confusion with nonmuscle cells.

Evaluation of the junctional conductance. The macroscopic gap junctional conductance (Gj) was determined using a dual-voltage clamp technique applied to myocyte cell pairs. Both cells of a pair were at first clamped at a common holding potential (Vh; close to −70 mV), then a pulse (e.g., −10 mV) was applied to one cell while the other was maintained at Vh, to generate a transjunctional voltage difference (Vj). Therefore, when cells in contact were connected by open junctional channels, this voltage gradient induced a junctional current (Ij) flowing through them from one cell to its neighbor and was compensated by an opposite current supplied by the feedback amplifier connected to the cell maintained at Vh. Gj was then calculated by dividing Ij by the amplitude of the Vj pulse. Current and potential records were digitized, stored, and analyzed with a personal computer by means of a software package (pCLAMP; Axon Instruments, Burlingame, CA). Gj was expressed as means ± SE.

Two recording configurations were used, either the conventional whole cell or the perforated patch configuration. In the latter situation, as previously described (32), after the gigaseal formation, the membrane patch under the pipette tip was permeabilized with amphotericin B, a pore-forming antibiotic, instead of being ruptured. Membrane pores made by amphotericin B (or the related compound nystatin) are not voltage dependent, are permeable to monovalent ions (something selective for monovalent cations over anions), and are impermeable to multivalent ions and molecules >0.8 nm in diameter. Therefore, this configuration allows the prevention of both the dilution of cytosolic components and the disruption of the normal intracellular Ca²⁺-buffering mechanisms.

The antibiotic, in the form of a 50-mg/ml stock solution of amphotericin B in DMSO, was added to the pipette filling solution to yield a final concentration of 150 μg/ml. After gigaseal was obtained, the access resistance dropped within 8 min to 20–40 MΩ, and experiments were started when the access resistance fell to 30 MΩ. On occasion, the perforated patch spontaneously converted into the whole cell configuration. This change was evident by the rapid activation of a large leak current in the related cell, together with a drop in Ij in the second cell.

Ca²⁺ imaging experiments. A possible involvement of a rise in the intracellular free Ca²⁺ concentration ([Ca²⁺ᵢ]) during amytalin A action was examined by photometry of the light emission of the fluorescent Ca²⁺ indicator indo 1. The use of this compound allows us to rule out the possibility of a partial loss of the Ca²⁺ indicator (through a permeabilized membrane or through incidental bleaching) that could mask an eventual increase of free Ca²⁺ concentration. Indeed, the ratio of the fluorescent signals from the free and from the Ca²⁺-bound molecules does not depend on dye loading or leakage, nor on cell thickness, and possible changes in [Ca²⁺ᵢ] during the experiment can be studied by determining the fluorescent signal ratio F₄₀₅/F₄₈₅.

Briefly, cells were loaded for 30 min at 37°C in the dark with 3 μM of the ester form of indo 1 (indo 1-AM, dissolved in DMSO) with the addition of 0.03% Pluronic acid F-127 (diluted in Tyrode solution) to facilitate the loading of the Ca²⁺ indicator. Cells were then washed off carefully several times with Tyrode to prevent further loading and were placed again in the cell incubator for 15 min to complete ester hydrolysis. Fluorescence was recorded using an epifluorescence inverted microscope (Olympus IX 70). Excitation was set in a range of 351–364 nm, and the fluorescent emission of both the free (405 nm) and Ca²⁺-bound (405 nm) forms of the dye were directed by a dichroic filter to two photomultiplier tubes.

Solutions. Gj measurements were made at room temperature (22–24°C) after replacing the culture medium with a Tyrode solution that contained (in mM) 144 NaCl, 5.4 KCl, 1.0 MgCl₂, 2.5 CaCl₂, 0.3 NaH₂PO₄, 5 HEPES, and 5.6 glucose (buffered to pH 7.4 with NaOH). The test solutions were applied to one cell while the other was maintained at Vh, with 300 μA of current flowing through them from one cell to its neighbor and was compensated by an opposite current supplied by the feedback amplifier connected to the cell maintained at Vh. Current and potential records were digitized, stored, and analyzed with a personal computer by means of a software package (pCLAMP; Axon Instruments, Burlingame, CA). Gj was expressed as means ± SE.
sitioned in the bath near the cell pair. Washing of the test fluids was similarly performed by switching to a Tyrode solution flowing out of a second capillary joined to the first at the opening. Low resistance (1.5–2.5 MΩ) patch pipettes were backfilled with a filtered solution that contained (in mM) 140 KCl, 0–5 MgATP, 5 EGTA, 10–15 glucose, 0.1 GTP, and 10 HEPES (buffered to pH 7.2 with KOH) and connected the cells to their respective feedback amplifiers (Biologic RK 300, Grenoble, France). H-7 and calyculin A were dissolved in DMSO before addition to bath solution. Antimycin A and cyclosporin A were dissolved in ethanol before addition to solutions, whereas okadaic acid (ammonium salt), heparin, I2, and p-nitrophenyl phosphate (Boehringer) were directly added to the solutions. All chemicals, unless otherwise stated, were obtained from Sigma.

RESULTS

The degree of cell-to-cell communication between cardiac cells is closely determined by the metabolic state of the cells. Both myocytes of the pair were clamped at $V_h$, then a 10-mV $V_j$ was applied; when cells were interconnected, this difference resulted in a current flowing through junctional channels, compensated by an opposite current supplied by the feedback amplifier connected to the cell maintained at the holding potential. In whole cell conditions, $G_j$ measured between neonatal rat cardiomyocytes was well maintained when experiments were carried out with 5 mM ATP in the patch pipette solution, whereas it rapidly decreased to complete closure of the channels within 12–20 min when ATP was absent (31). At a relatively low ATP concentration (1 mM), $G_j$ was progressively reduced, as illustrated in Fig. 1A, to ~12.5% of its initial value after 15 min. The degree of GJIC after 5, 10, and 15 min of recording was compared when pipette filling solutions contained, respectively, 0, 1, 2, and 5 mM ATP and were expressed in percent of the original conductances (Fig. 1B). ATP at 2 mM allowed almost the whole level of cell-to-cell communication to be retained.

In intact cardiac myocytes, under physiological conditions, the intracellular ATP concentration is kept at a level ranging from 3.0 to 7.5 mM (1). To examine whether, in vivo, the depletion of ATP expected from an inhibition of mitochondrial respiration leads to an inhibition of GJIC, the consequences of exposure of the cells to the respiratory inhibitor antimycin A (10 μM) were examined (Fig. 2A) in double-perforated patch conditions to avoid cell dialysis by pipette solution. When cell pairs were exposed to antimycin A, the currents elicited by $V_j$ progressively decreased to complete interruption of the GJIC.

Gap junctional permeability is reduced by high increases in cytosolic Ca$^{2+}$ concentration, and it was suggested that such increases were responsible for the loss of GJIC between astrocytes observed after prolonged exposures (16 h) to antimycin A (5 ng/ml) (30). The possible involvement of this mechanism, in the short term observed in the present study, has been examined by monitoring the intracellular Ca$^{2+}$ level as the evolution of the ratio of indo 1 intensities at, respectively, 405 nm and 485 nm ($F_{405}/F_{485}$). Antimycin A application (10 μM), as shown in Fig. 2B, resulted in a substantial increase of the fluorescence signal ratio, increased from 0.56 ± 0.09 (mean ± SD) in control conditions to 1.05 ± 0.10 ($n = 13$) during drug treatment. The efficiency of the Ca$^{2+}$-detection technique was then tested, in the range corresponding to the activation of contractile activity, by applying a high-K$^+$ solution (100 mM) that was able to increase the $F_{405}/F_{485}$ fluorescence ratio (Fig. 2B) to a higher ratio (1.19 ± 0.11; $n = 13$). Because it has been previously shown that in these cells the intercellular diffusion of a fluorescent dye was virtually unmodified during exposure to high-K$^+$ solutions (32), the increase in cytosolic Ca$^{2+}$ concentration observed during antimycin A treat-
ment does not seem responsible for the interruption of GJIC. A possible involvement of a rise in intracellular H+ concentration in the antimycin A effect is also made unlikely by the observation of intercellular uncoupling in patch-clamp conditions when intracellular pH is strongly buffered.

The significant decrease in ATP concentration reported during antimycin A treatment (30), as all other treatments employed with the view to impede the protein phosphorylation pathway (ATP removal by intracellular dialysis or PK inhibition), appears to lead to a closure of the gap junctional channels because of the permanent tonic activity of membrane-bound protein PP(s), but the enzyme responsible for this channel closing remains to be identified. The lack of influence of Mg2+ on the activity of this enzyme is not in favor of the involvement of the Mg2+-dependent PP2C in the loss of junctional channel activity (31). Therefore, the effects of inhibitors or activators of enzymatic activities of other members of the phosphoserine/threonine PP family were examined.

Cyclosporin A failed to prevent the decay of GJIC induced by nonspecific inhibition of PK, whereas okadaic acid or calyculin A allowed the preservation of the main part of cell-to-cell communication. Cruciani et al. (9) assessed the PP activities in hamster V79 fibroblasts expressing Cx43 and studied the effects of different PP inhibitors. The Ca2+-dependent PP2B (calcineurin) was found in a considerable amount in the cells, and PP2B inhibitors delayed the normalization of GJIC in 12-O-tetradecanoylphorbol 13-acetate (TPA)-treated cells. The possible involvement of PP2B in the rundown of Gi of rat cardiac myocytes in ATP-deprived conditions was examined with a PP2B inhibitor, cyclosporin A (1 μM). As illustrated in Fig. 3, the complete interruption of GJIC was not prevented by the presence of cyclosporin A.

The evolutions of Gi in ATP-deprived conditions were then observed in the presence of okadaic acid or calyculin A, which are both potent inhibitors of the catalytic subunits of PP1 and PP2A in vitro, but with different effectiveness. In these conditions, calyculin A has a 50% inhibition value in the nanomolar range. At these concentrations, okadaic acid inhibits PP2A but is a 100-fold less potent inhibitor of PP1 than calyculin A (20). As shown in Fig. 4A, at a concentration (1 μM) expected not to affect PP2B (17), okadaic acid markedly slowed down the fading of Gi in ATP-free conditions, and a substantial cell-to-cell communication was still present after 13 min (52 ± 12%). However, an even better preservation of junctional coupling was obtained in the presence of calyculin A (0.1 μM, Fig. 4B), which substantially delayed the loss of channel activities, since 90% of the original conductance was still recorded after ~7.5 min. Thereafter, the degree of intercellular communication slowly decreased with time.
The capacity of both okadaic acid and calyculin A to markedly delay the progressive closure of gap junctional channels elicited by ATP deprivation leads to the possibility that PP2A, or more plausibly, PP1, might be responsible for the dephosphorylating process leading to the interruption of GJIC.

PP1 inhibitors prevent the rundown of junctional conductance in ATP-deprived conditions. Heparin, a sulfated polysaccharide, inhibits different PKs, including casein kinase 2 (14) as well as the dephosphorylation of different proteins by PP1 (11). In the absence of ATP, phosphorylation by PKs are interrupted, and heparin (100 µg/ml) was used, in these conditions, as a PP1 inhibitor. In its presence, the degree of GJIC slightly decreased with time, but an important intercommunication persisted (77 ± 15% of the initial Gj after 5 min) in ATP-derived conditions (Fig. 5A). Thus the inhibition of PP1 activities allowed an important proportion of junctional channels to stay open in spite of the interruption of phosphorylating reactions.

PP inhibitor 2 (I2) is one of the endogenous regulators of PP1 activity, which forms with the PP1 catalytic subunit a stable and inactive complex that requires glycogen synthase kinase 3 and Mg-ATP for reactivation (for review, see Ref. 27). Because such reactivation cannot occur in these ATP-deprived conditions, I2 specifically interrupts reactions catalyzed by PP1. As shown in Fig. 5B, when I2 (100 nM) was added to the ATP-free solution, the loss of activity of junctional channels was considerably slowed down. When whole cell conditions were maintained for a longer period (in 2 pairs of cells), 55 ± 11% of the original conductance was still measured after 24 min. In conclusion, both heparin and PPI2 substantially prevented the loss of channel activity triggered by ATP removal, showing that the PP1 inhibition (perhaps still partial) allows the prevention of the closure of junctional channels.

Inhibitions of both the phosphorylation and PP1 pathways preserved cell-to-cell communication. In the presence of ATP, Gj was progressively reduced to a...
stable level corresponding to ~13% of its initial value within 10 min by H-7, a broad spectrum inhibitor of serine/threonine PKs, used at a relatively high concentration (1 mM) with the intent to inhibit a range of PKs (31). H-7, although structurally unrelated to ATP, is considered to compete with ATP for free enzymes (18). When both heparin (100 μg/ml) and H-7 (1 mM) were present in the external bath, the degree of intercellular communication remained almost unmodified, as shown Fig. 6. The ability of a PP inhibitor to prevent H-7 effects allows the possibility of other mechanisms of action of H-7 (e.g., a deleterious effect on membranes or cytoskeleton) to be excluded and shows that this compound alters the junctional conductance by impeding the phosphorylation pathway. It also shows that the simultaneous hindrance of both phosphorylation and dephosphorylation allows preservation of cell-to-cell communication, at least for the duration of the experiment.

Stimulation of PP1 activity triggers the interruption of cell-to-cell communication despite the presence of ATP. With the aim of confirming that PP1 activity was responsible for the closure of junctional channels when protein phosphorylation was depressed, the effects of the enhancement of PP1 activity have been examined by stimulation of the endogenous PP1 activity. The compound p-nitrophenyl phosphate, able to specifically stimulate PP1 activity (12) although it acts as a diversion substrate for other PPs (27), appears to be a suitable tool for the identification of the active PP form. The addition of p-nitrophenyl phosphate (1 mM, Fig. 7A) to the ATP-containing solution that filled the patch pipettes induced a progressive fading of the junctional currents.

To ascertain that p-nitrophenyl phosphate effects were not a consequence of a nonspecific action, this drug was added with I2 (100 nM) with the intention to prevent the enhancement of endogenous PP1 activity. In these conditions, the effects of p-nitrophenyl phosphate exposure were markedly slowed down (Fig. 7B).

**DISCUSSION**

The $G_j$ measured among neonatal rat cardiomyocytes in conventional double whole cell mode was well maintained when ATP was present in the patch pipette solution, whereas it rapidly vanished to complete interruption of the GJIC if ATP was absent. An ATP intracellular concentration of 2 mM allowed the preservation of a stable level of cell-to-cell communication. When $G_j$ between paired cardiomyocytes was determined after excision of one of the cells, ATP had to be present in the bath solution in a comparable concentration level to preserve cell-to-cell communication, but Sugiura et al. (28) suggested that $G_j$ was regulated.
through a specific ligand-receptor interaction between ATP and gap junctional proteins rather than through the promotion of protein phosphorylation.

When in vivo ATP synthesis was interrupted by exposure to antimycin A, an inhibitor of mitochondrial respiration, the GJIC was completely abolished. Prolonged exposure (16 h) to antimycin A also interrupted the cell-to-cell diffusion of Lucifer yellow between rat astrocytes, and this effect was interpreted as a consequence of an increase in cytosolic Ca\(^{2+}\) concentration (30). Antimycin A is, indeed, considered to deplete mitochondrial Ca\(^{2+}\) (35). In the present study, short-time treatments elicited a relatively important increase in [Ca\(^{2+}\)]\(_i\), that, however, remained less important than the rise observed when the cells were exposed to a high-K\(^+\) solution, a treatment known to have no effect on the cell-to-cell diffusion of a fluorescent dye in these cells (32).

In ventricular myocytes of neonatal rats, several observations (the effects of ATP replacement by some other nucleotides, of broad-spectrum inhibition of endogenous serine/threonine PKs, and of introduction into the cells of a nonspecific exogenous PP) led to the possibility that the presence of high energy nucleotides is essential to allow PK activities to counteract the tonic activities of endogenous PP(s) (31). Although several PKs have an established role in this process, less is known about the involvement of PPs. The four main phosphoserine/threonine protein phosphatases, PP1, PP2A, PP2B, and PP2C, have been previously detected in heart (for review see Ref. 27). The main aim of the present study was to identify the dephosphorylating enzyme whose activity is responsible for the interruption of cell-to-cell communication among rat ventricular myocytes.

The lack of influence of Mg\(^{2+}\) on the loss of channel activity is not in favor of the involvement of PP2C in the rundown of \(I_j\) (31). Cyclosporin A, a calcineurin (PP2B) inhibitor, was able to delay the gap junction plaque recovery of hamster V79 fibroblasts expressing Cx43 after TPA exposure (9); however, in the present study, it did not prevent the rundown of \(I_j\) occurring in ATP-deprived conditions.

Several works have suggested that PP1 and/or PP2A are the PPs involved in the regulation of gap junctional communication. Exposure of rat liver epithelial cells to 18β-glycyrrhetinic acid rapidly and reversibly elicited an interruption of cell-to-cell communication together with a Cx43 dephosphorylation, unless okadaic acid or calyculin A, inhibitors of PP1 and PP2A, were present (13). A part of the junctional uncoupling action of BDM seemed to result from a dephosphorylation process (32), and it was suggested (38) that this compound could enhance the activity of endogenous phosphatases PP2A, and, to a lesser extent, PP1. In Madin-Darby canine kidney cells, okadaic acid pretreatment (60 nM) potentiated the TPA-induced increase in phosphorylated Cx43, suggesting that okadaic acid-sensitive PP(s) participate in the dephosphorylation of Cx43 (2). In a communication-deficient cell line (SKHep1) transfected with a cDNA encoding human Cx43, okadaic acid (300 nM) shifted the frequency histograms of unitary conductances in the same direction as phosphorylating agents such as TPA, 8-bromoadenosine 3’5’ cyclic monophosphate, or forskolin (24), suggesting that the gating properties of gap junctional channels would depend on PP1 and/or PP2A activities.

In the present study, okadaic acid slowed down the decay of \(G_j\) in ATP-deprived conditions but was less efficient than calyculin A to preserve the cell-to-cell coupling, suggesting that PP1 might be the main protein phosphatase involved in the modulation of the channel. The effectiveness of two selective PP1 inhibitors, I2 and heparin, to preserve cell-to-cell coupling in ATP-deprived conditions has been examined. I2 is a heat- and acid-stable 22.8-kDa protein that specifically inhibits PP1 in vitro (6, 27) as well as in intact cells (10). Heparin is known to almost completely inhibit PP1, particularly the membrane and nuclear fractions (5). Both of these compounds allowed, in whole cell conditions, the preservation of cell-to-cell communication in the absence of ATP. Similarly, when the phosphorylation pathway was in the presence of ATP, hindered by H-7, the degree of cell-to-cell coupling remained almost unaltered, provided that heparin was present.

To verify that PP1 activity is responsible for the closure of gap junctional channels, we examined the consequences of its selective activation by p-nitrophenyl phosphate in the presence of ATP and verified that this treatment led to a complete interruption of the cell-to-cell communication except when I2 was concomitantly added to selectively prevent the effects of PP1 activation. The activity of junctional channels of rat ventricular myocytes appears regulated by ongoing phosphorylation/dephosphorylation under basal conditions, and PP1 activity leads to the rapid closure of all intercellular channels when it is not counterbalanced by PK activities. PP1 is a broad specificity phosphatase, able to regulate many biological processes, including, for example, synaptic plasticity, cell cycle, gene transcription, glycogen metabolism, and muscle contraction (for review see Ref. 27).

It has also been reported that this enzyme can modulate the activity of a number of membrane channels, including, for example, that of voltage-gated Na\(^+\) (26), Ca\(^{2+}\) (N- and P-types, see Ref. 29), or K\(^+\) (outward K\(^+\), see Ref. 10) channels as well as that of a Ca\(^{2+}\)-sensitive nonspecific cation channel (36) or glutamate receptor channels, including N-methyl-D-aspartate (4, 34) or Dl-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (37) receptors.

Cx43, as with a large majority of cell proteins, is predominantly phosphorylated on serine residues, and important changes in its state of phosphorylation can be resolved by differences in electrophoretic mobilities. Preliminary results showed that several treatments aimed to shift the protein phosphorylation/dephosphorylation balance toward dephosphorylation reduced the degree of junctional coupling of cardiac myocytes without modifying the extent of Cx43 phosphorylation examined by Western blot analysis (9a).
Similar observations have been reported in other Cx43-expressing cells, such as T51B rat liver epithelial cells (19), V79 hamster fibroblasts (9), or rat astrocytes (23). Limited changes in the degree of phosphorylation of Cx43, particularly on tyrosine residues, may, however, occur without shift of the electrophoretic mobility of the protein. Sustained disruptions of GJIC have, indeed, been found to be correlated with an enhanced Cx43 phosphorylation on tyrosine (for review, see Ref. 22). In the present study, an enhanced phosphorylation is not expected in ATP-depleted conditions. On the other hand, the tyrosine kinase inhibitor genistein had no effect on the junctional coupling of these cells (33).

In conclusion, the activity of junctional channels of rat ventricular myocytes appears to be regulated by ongoing phosphorylation/dephosphorylation under basal conditions, and PP1 activity leads to a rapid interruption of the intercellular communication if PK activities do not balance its action. Constitutively active PP1, plausibly anchored in the vicinity of junctional channels, might then be a target in signaling pathways for many extracellular molecules (e.g., growth factors, cytokines, and neurotransmitters), allowing modulation of the degree of cell-to-cell communication.

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