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ATP release by way of connexin 36 hemichannels mediates ischemic tolerance *in vitro*

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Abstract

Spreading depression (SD) is a self-propagating wave of neuronal and glial depolarization that may occur in virtually any gray matter region in the brain. One consequence of SD is an increased tolerance to ischemia. It has been shown that during cortical SD ATP is released into the extracellular space and activation of purinergic receptors leads to the induction of ischemic tolerance. In the present study we show that depolarization of cultured neurons induces ischemic tolerance which is mediated by purinergic receptor activation. Depolarization causes the release of ATP into the extracellular medium, which may be prevented by treatment with the connexin hemichannel blockers flufenamic acid and quinine, but not the pannexin hemichannel blocker carbenoxolone. Knockdown of connexin 36 expression by siRNA greatly reduces the amount of ATP released during depolarization and the subsequent degree of ischemic tolerance. We conclude that during depolarization neurons release ATP by way of connexin 36 hemichannels.

Keywords: Cortical spreading depression; ATP; Connexin hemichannels; Depolarization; Ischemia; Purinergic receptors

The resistance of the brain to ischemic injury may be transiently increased by exposure to a non-injurious preconditioning stimulus. It has been shown that ischemic tolerance reprograms the response to ischemia, leading to a protected state [1]. Cortical spreading depression (CSD) is a well studied phenomenon in the brain that induces a substantial degree of ischemic tolerance [2–4]. CSD is described as a slowly propagating wave of depolarized neurons and glia that causes depressed synaptic activity [5–7]. CSD results in transmembrane redistributions of K^+, glutamate, Ca^{2+}, Na^+, and Cl^-, which further lead to cell swelling and a reduction in the extracellular space. The induction of CSD has been shown to occur *in vivo* in the setting of epileptic seizures [8], hypoxia [9], traumatic brain injury [10], focal ischemia [11], and migraine [12]. Experimental induction of CSD is accomplished with the use of mechanical trauma, electrical stimulation, or the application of high potassium or a variety of other chemical agents [13].

In the brain ATP is known to act as a signaling molecule where it may influence cellular proliferation and differentiation [14,15]. ATP also modulates the excitability of neurons [16] and is known to alter gene expression [17]. Two families of purinergic receptors, ligand gated cation channels (P2X) and G-protein coupled receptors (P2Y), mediate the effects of extracellular ATP. There are a variety of ways that ATP may be released in the brain including ruptured cell membranes, vesicular release from nerve terminals and passage through an assortment of channels including voltage-dependent anion channels [18], the cystic fibrosis transmembrane conductance regulator, CFTR [19], P2X7 receptor-channels [20], connexin 43 hemichannels [21–23], connexin 32 hemichannels [24], and pannexin I hemichannels [25].

We have previously reported that during CSD ATP is released into the extracellular space and the activation of

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purinergic receptors contributes to the induction of ischemic tolerance [26]. As well, in cultured rat primary cortical neurons, exposure to extracellular ATP provides protection against potassium cyanide, a chemical form of hypoxia, and oxygen glucose deprivation (OGD).

Previous studies have implicated a role for connexins in various models of preconditioning and ischemic injury. Connexin 43 hemichannels have been implicated in the induction of ischemic tolerance in the heart [27] and Naus et al. [28] found that blocking gap junctions during exposure to glutamate in co-cultures of astrocytes and neurons resulted in increased neuronal injury. These authors also report that connexin 43 heterozygous null mice show a significantly larger infarct volume following focal ischemia in the brain. Connexin 36 forms a neuron specific channel in the brain. When these channels are blocked with various pharmacological agents such as quinine, quinidine and mefloquine the progression of CSD waves is inhibited [29]. In the present study, we show that potassium chloride (KCl) induced depolarization results in the release of ATP from cultured cells by way of connexin 36 hemichannels and leads to protection against ischemia *in vitro*. We also show that activation of the P2Y purinergic receptors as well as induction of the PKA and PLC signal transduction pathways are required for the induction of ischemic tolerance.

Materials and methods

All surgical procedures followed the guidelines of the Canadian Council for Animal Care and were approved by the Animal Care Committee of the University of Ottawa.

Primary cortical neuron cultures. Pregnant Sprague–Dawley rats (Charles River Canada, St. Constant, QC, Canada) at E-15/16 were anesthetized with halothane and sacrificed by cervical dislocation. Fetuses were decapitated and the cortical region dissected out and collected under sterile conditions. Primary cortical neurons were cultured as described

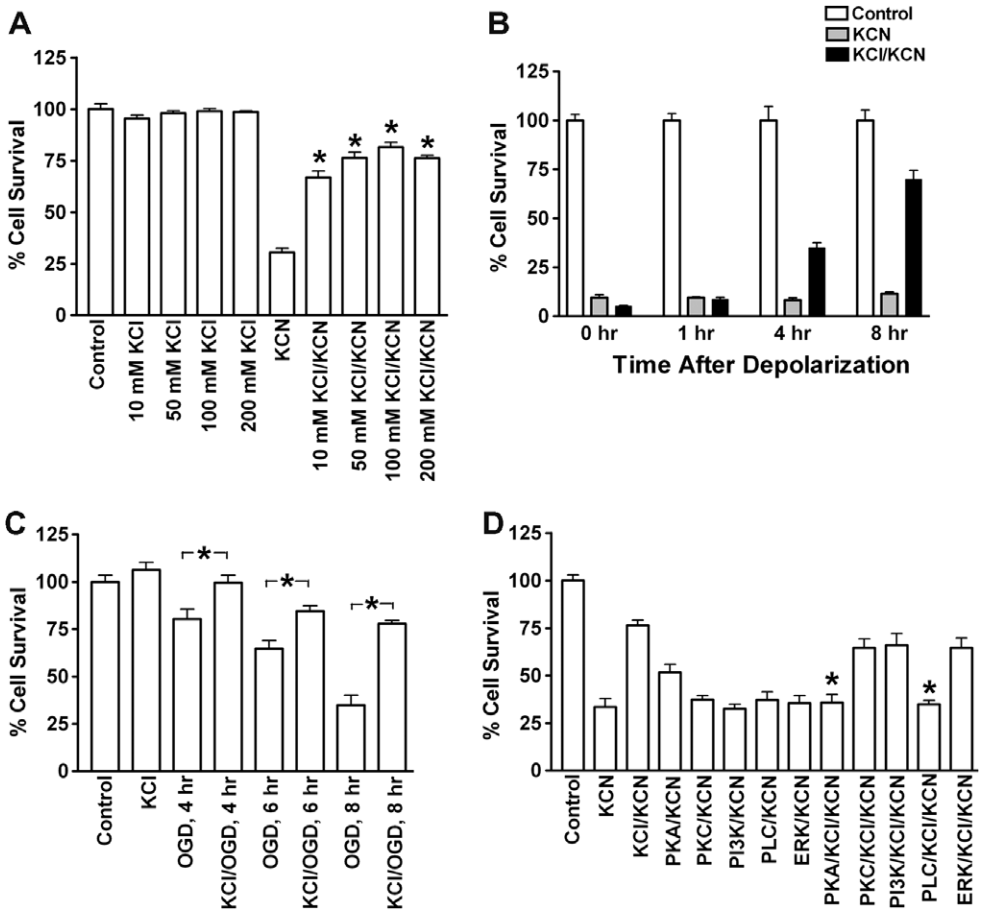

Fig. 1. Induction of tolerance to *in vitro* forms of hypoxia by depolarization. (A) Primary cortical cultures were exposed to elevated KCl for 30 min at the concentrations indicated. After 8 h, cells were exposed to 1 mM KCN for 4 h. Cell survival was determined 24 h later. All concentrations of KCl provided significant protection against KCN with 100 mM being the most significant. (B) Induction of tolerance requires time to develop, no protection was seen at 0 and 1 h following pretreatment, maximal protection was seen 8 h following exposure to KCl. (C) Exposure to 100 mM KCl provided protection against oxygen glucose deprivation. (D) Signal transduction pathways mediating the induction of tolerance to metabolic hypoxia. Cultures were exposed to KCl (100 mM for 30 min) alone, or in the presence of a signal transduction inhibitor, and returned to normal medium for 8 h before exposure to KCN (1 mM for 4 h). Cell survival was determined 24 h later. Inhibition of PKA or PLC almost completely eliminates tolerance, whereas inhibition of the PI3 kinase, ERK or PKA pathways did not show a significant inhibition of protection. A star indicates a statistically significant difference from KCl/KCN treatment alone. Cultures were also exposed to KCN (1 mM for 4 h) alone, or in the presence of an inhibitor. $N = 4$.

previously [26]. The cells were plated on poly-D-lysine (100 μg/ml, Sigma) dishes in neurobasal medium (Invitrogen) containing N2 and B27 supplements (Invitrogen) and kept for 7 days *in vitro* (DIV) without replacing the culture medium in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. Cultures were grown for another 6–7 days, with one half of the medium being changed every 2–3 days, before being used in an experiment.

Cerebellar granule neuron cultures. Cerebellar granule neurons (CGN) were isolated from 7- to 9-day-old mice, plated and cultured as described previously [30] on poly-D-lysine (20 μg/ml; PDL)-coated surfaces in neurobasal medium (Invitrogen) containing N2 and B27 supplements (Invitrogen). Cells were plated on poly-D-lysine coated plates at the same densities as stated above.

Treatment of cultures. Cultures of rat primary cortical neurons were treated with 100 mM KCl for 30 min and returned to normal medium for up to 8 h. They were then exposed to oxygen glucose deprivation (OGD, 8 h) or potassium cyanide (KCN, 1 mM, 4 h) and returned to normal medium. Cell survival was determined 24 h by LDH assay. OGD was carried out in a hypoxic glove box (Coy Laboratory Instruments, Inc., Grass Lake, Michigan). Metabolic hypoxia (KCN) was chosen as a model to further characterize the protective effects of KCl and identify the signal transduction pathways involved. Unless otherwise stated, cultures were pretreated with the compound being tested for 1 h and then exposed to KCl (100 mM) in the presence of the compound for 30 min. Cultures were then returned to normal medium for 8 h before exposure to KCN (1 mM) for 4 h. Cultures were returned to normal medium and the extent of cell survival determined 24 h later by measurement of lactate dehydrogenase (LDH) activity released in the media. The LDH assay was performed using the CytoTox96 Non-Radioactive Cytotoxicity Assay Kit (Promega) according to the manufacturer's protocol. Cytotoxicity was determined by measuring wavelength absorbance at 490 nm. Ischemia induced LDH release was expressed as a percentage of experimental LDH release/maximal LDH release.

ATP measurements. ATP release from cells was measured using the Adenosine 5′-triphosphate Bioluminescent Assay Kit (Sigma Aldrich) according to the manufacturer's instructions. Samples were read using the SpectraMax luminometer (Molecular Devices). A standard curve was performed prior to all experiments.

siRNA transfections. Connexin 36 siRNA was purchased from Santa Cruz Biotechnology containing three target specific sequences. About 10 pmol of siRNAs were transfected into CGN cultures using Lipofectamine 2000 according to the manufacturer's instructions. Downregulation and quantitation of connexin 36 mRNA was evaluated 24 h post-transfection using RT-PCR as described previously [17] with the following primers: Cx36 sense (5′-TGTCAATGGGGTGCTCCAGA-3′) and Cx36 antisense (5′-TCTGCCTTGGGGCTACTTGC-3′). The primers for GAPDH were: GAPDH sense (5′-CATGGCCTTCCGTGTTCCTACCC-3′) and GAPDH antisense (5′-CTTCGGCCGCCTGCTTCA-3′).

Statistics. Statistical significance was determined using the Student's *t*-test of significance of difference of means. Error bars indicate standard deviation.

Results and discussion

Potassium chloride protects against in vitro models of ischemia

To determine the protective effects of depolarization in primary cortical neurons, cultures were exposed to various concentrations of potassium chloride (KCl) in normal medium. At the concentrations used KCl alone did not induce any cell death or proliferation (Fig. 1A). To precondition the cells, cultures were exposed to elevated KCl for 30 min and returned to normal medium for 8 h [26]. Following this the cells were exposed to potassium cyanide (KCN) for 4 h. All concentrations tested provided protection against KCN, but 100 mM provided the greatest degree of protection and was the concentration used throughout the duration of this study (Fig. 1A). When cultures were treated with KCN immediately following exposure to KCl for 30 min, there was no significant protective effect. However, if cultures were returned to normal medium for a period of time before exposure to KCN the protective effect was maximal after 8 h (Fig. 1B). Cultures were also exposed to oxygen glucose deprivation for various times to further characterize the protection by KCl induced depolarization. Cells were pretreated with 100 mM KCl for

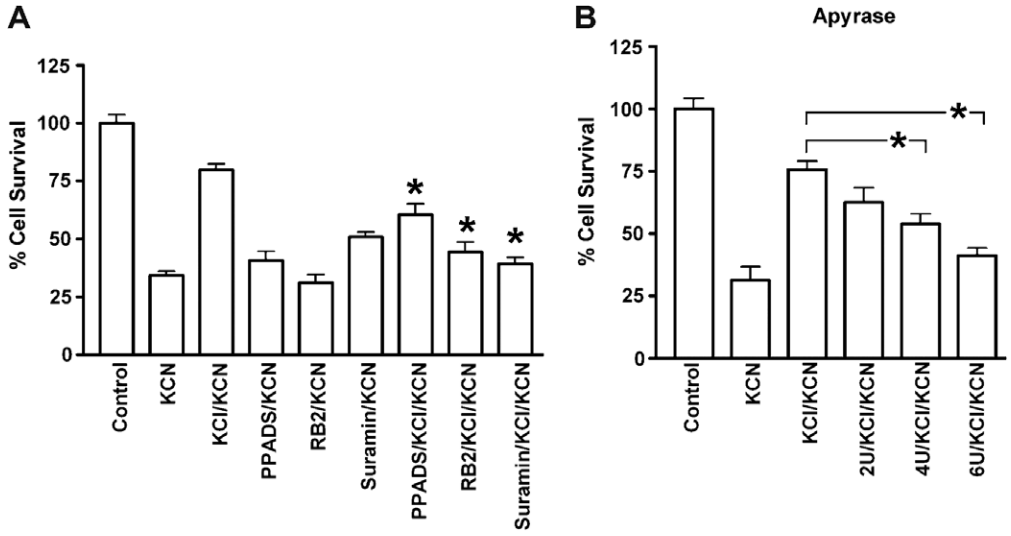

Fig. 2. Protection afforded by KCl is in part due to the release of ATP and the activation of purinergic receptors. (A) Pretreatment with purinergic receptor antagonists reduce the protection elicited by KCl. Suramin, a general purinergic receptor antagonist, blocks the protection induced by KCl. The P2X antagonist, PPADS, slightly but significantly blocks protection whereas the P2Y receptor inhibitor, RB2, almost completely blocks protection. (B) Application of apyrase, an enzyme that hydrolyzes ATP to AMP, 30 min before treatment with KCl reduced protection in a dose-dependent manner. $N = 4$.

30 min and returned to normal media for 8 h. Cells were then exposed to OGD for either 4, 6, or 8 h. A significant increase in cell survival was also observed in this model of ischemia for all OGD conditions with the greatest significance occurring at the 8 h OGD exposure (Fig. 1C). Since the results for the OGD insult were comparable to that of the KCN insult, in subsequent experiments KCN was used to induce ischemic cell death for reasons of convenience.

The PLC and PKA signal transduction pathways play a role in the induction of ischemic tolerance by depolarization

To identify signal transduction pathways that may be involved in the induction of tolerance by KCl induced depolarization, primary cortical neurons were treated with various kinase inhibitors for 1 h prior to and during exposure to 100 mM KCl for 30 min. The cultures were then returned to normal medium for 8 h before exposure to 1 mM KCN for 4 h. Cell survival was determined 24 h later. Inhibition of MEK 1/2, PKC and PI3K signal transduction pathways did not significantly affect cell survival (Fig. 1D). Inhibition of the PKA pathway with Rp-cAMP reduced cell survival from about 76% to about 35%. As well, inhibition of the PLC pathway with U73122 also caused a decrease in cell survival of 39% (Fig. 1D). These results implicate a role for both the PLC and PKA pathways in the induction of tolerance by KCl induced depolarization. In previous experiments the PLC and PKA signal transduction pathways were also shown to be involved in the onset of protection against KCN induced cell death by application of extracellular ATP [26].

ATP is involved in the induction of ischemic tolerance by KCl depolarization though activation of P2 Y purinergic receptors

To test the hypothesis that purinergic receptor activation is mediating the induction of tolerance by KCl depolarization, primary cortical cultures were treated with either suramin, a general P2 receptor antagonist, PPADS, a P2X receptor antagonist, or reactive blue 2 (RB2), a

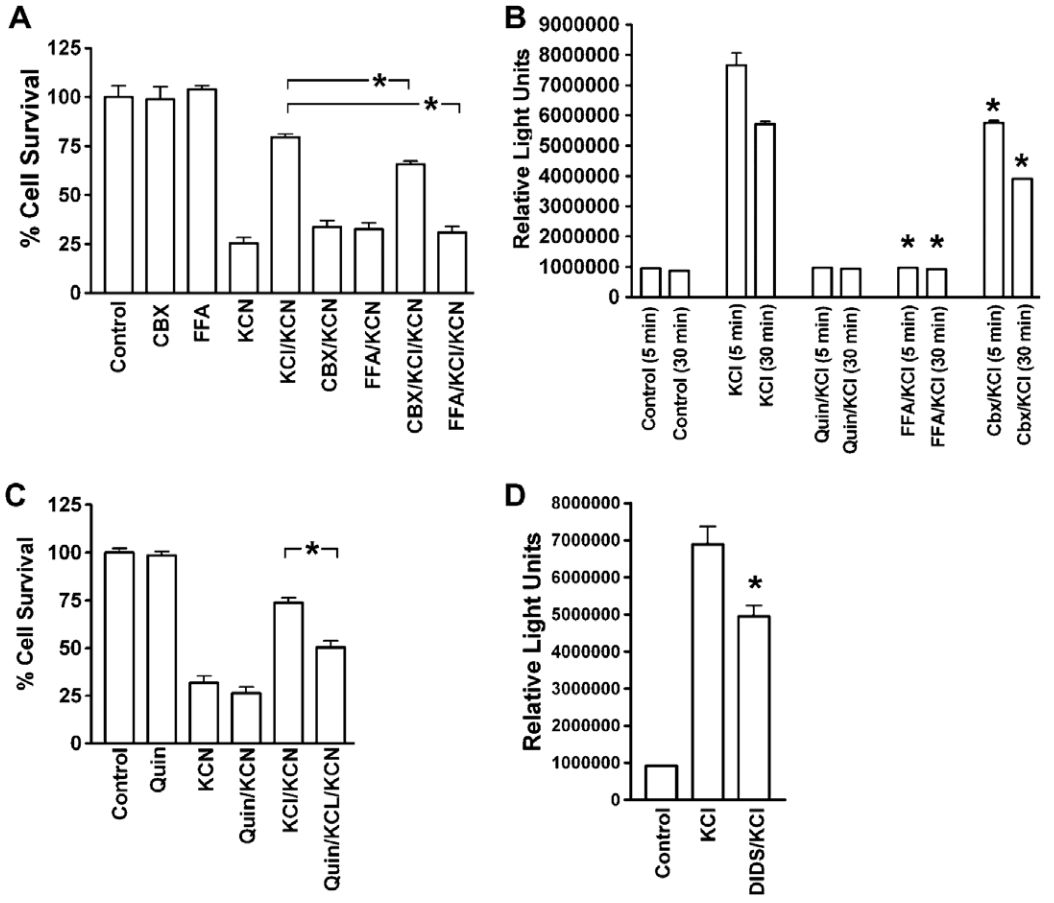

Fig. 3. Exposure to KCl causes the release of ATP though connexin hemichannels. (A) Primary cortical cultures were exposed to either CBX (50 μM), an inhibitor of pannexin channels, or FFA (50 μM), an inhibitor of connexin channels for 30 min prior to exposure to 100 mM KCl for 30 min. Cells were returned to normal media for 8 h before exposure to 1 mM KCN for 4 h. Results show that both FFA and CBX significantly block the protection elicited by KCl pretreatment, although FFA is much more effective. (B) Direct measurement of extracellular ATP released by cortical neuron cultures in response to KCl induced depolarization. Results show that there is a 7-fold increase in relative light units (RLU) in KCl treated cultures compared to controls. This increase is blocked by FFA and quin and to a slight degree Cbx. (C) Cells were pretreated with quinine (quin) (50 μM), an inhibitor of connexin 36 channels, and then treated as above. Results show that quin significantly blocks the protection elicited by KCl. (D) DIDS, a chloride channel inhibitor, also slightly but significantly reduced the RLU compared to KCl treated cultures alone. $N = 4$.

P2Y receptor antagonist, for 1 h prior to and during exposure to KCl. Cells were returned to normal medium for 8 h and then exposed to 1 mM KCN for 4 h and the extent of cell survival was assessed 24 h later. Suramin (10 μM) reduced cell survival from 80% to 39% (Fig. 2A). When cultures were exposed to PPADS, there was a significant reduction in survival of about 20%, indicating that P2X receptor activation is involved in KCl induced protection. RB2 caused the protective effect of KCl to be reduced 36% also indicating activation of these receptors during the induction of tolerance (Fig. 2A).

In order to determine the involvement of ATP in depolarization induced tolerance, primary cortical neurons were pretreated with apyrase at various concentrations prior to and during application of 100 mM KCl for 30 min. A dose-dependent decrease in protection induced by exposure to KCl was observed with the most significant loss in protection corresponding to the highest concentration of apyrase applied (Fig. 2B). Direct measurement of ATP release during KCl induced depolarization revealed levels of extracellular ATP approximately 7-fold higher than in control cultures. According to the standard curve performed, extracellular ATP levels in control cultures were in the range of 1–10 nM whereas KCl treated cultures had an extracellular ATP concentration in the range of 100 nM–1 μM (Fig. 3C), leading to an approximate release of 225 fmol per cell in a 24 well plate containing 200 μL of medium.

Connexin channels release ATP during KCl induced depolarization

To determine how ATP is being released during KCl induced depolarization, primary cortical neurons were treated with the pannexin channel blocker, carbenoxolone (CBX), or the connexin channel blocker, flufluemic acid (FFA), 1 h prior to and during treatment with 100 mM KCl for 30 min. Cultures were returned to normal medium for 8 h before the addition of KCN for 4 h. Cell survival was assessed 24 h later. Exposure to CBX prior to and during depolarization results in a slight but significant reduction in cell survival. However, exposure to FFA almost completely eliminates the protection elicited by KCl, from about 79% survival to 30% (Fig. 3A). As

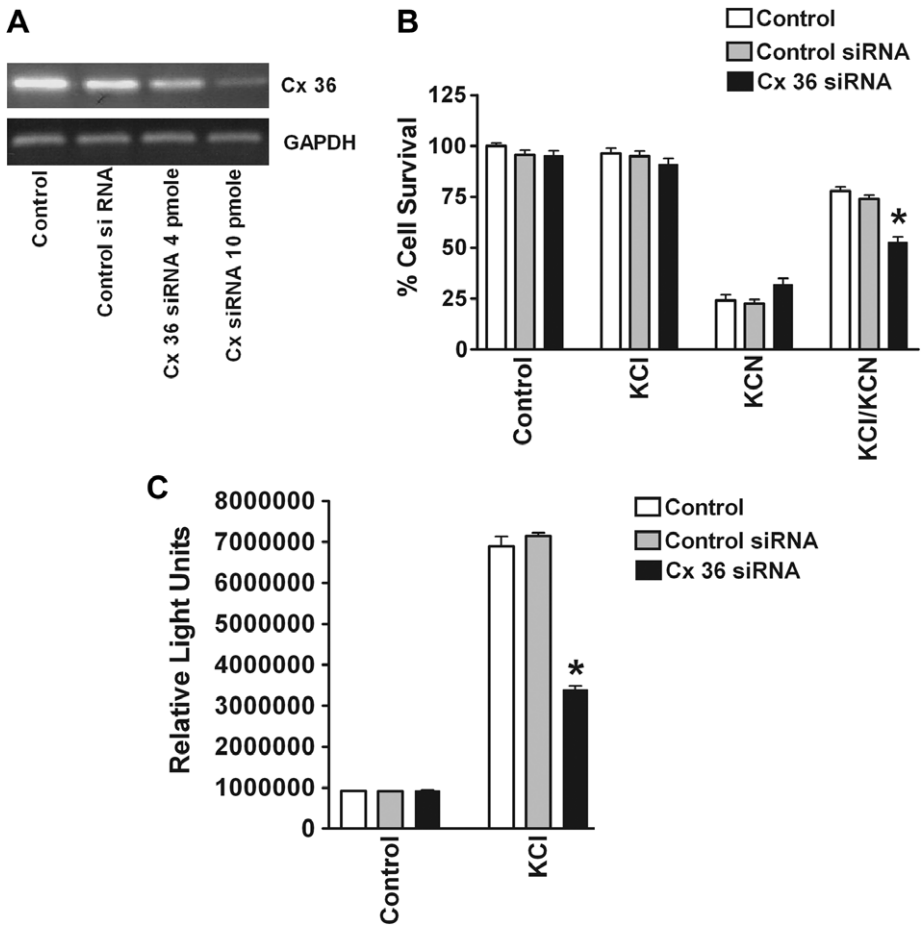

Fig. 4. ATP is released through connexin 36 hemichannels in response to depolarization. (A) RT-PCR showing the reduction in Cx36 mRNA in siRNA transfected CGN cultures. GAPDH was used as a loading control. (B) Cell survival assay showing the reduction in protection in Cx36 siRNA transfected cells compared to control siRNA transfected cells. (C) Luciferase measurement of extracellular ATP also showed that transfection of CGN cultures with Cx36 siRNA reduced ATP release in response to depolarization.

well, CBX exposure causes a slight but significant reduction in the release of ATP from KCl treated neurons, while FFA causes a reduction to control levels in the treated cultures (Fig. 3B). To determine the involvement of connexin hemichannels in ATP release cultured neurons were exposed to the connexin channel blocker quinine (Quin), which has been shown to specifically block Cx36 channels [31]. Exposure to Quin caused a 25% reduction in protection elicited by depolarization (Fig. 3C), as well as completely reducing the release of ATP to that of control levels (Fig. 3B). A standard curve was performed in the presence of Quin and compared to the normal standard curve. Since Quin is also know to inhibit chloride channels, the chloride channel blocker 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid (DIDS) was used to assess the role of these channels in the release of ATP by KCl induced depolarization. DIDS caused a slight but significant reduction in ATP release in KCl treated cultures (Fig. 3D). These results indicate that during depolarization ATP is being released into the extracellular space mainly via connexin hemichannels. They also indicate that some ATP may pass through pannexin hemichannels and chloride channels.

Connexin 36 is the main channel releasing ATP during depolarization

To further determine a role for Cx36 in the release of ATP during depolarization we made use of Cx36 specific siRNA in CGN cultures. Expression of Cx36 mRNA was almost completely abolished following siRNA transfection, whereas no effect was observed in siRNA control treated cultures (Fig. 4A). In addition, a 25% reduction in survival was observed in the absence of Cx36 (Fig. 4B), suggesting a role for Cx36 in KCl-mediated neuroprotection in cultured cells. As well, in Cx36 reduced cells, there was a significant reduction in the release of ATP in response to KCl compared to control siRNA treated cultures (Fig. 4C). However, there is not a complete reduction in protection or in ATP release from the Cx36 siRNA transfected cells. This may be due to the fact that Cx36 expression was not completely eliminated by the siRNA method. Also, ATP may be able to pass through some other type of channel such as those formed by other connexins or pannexin I or a chloride channel.

Taken together our study suggests that depolarization of neurons induces a degree of tolerance against KCN insult or OGD *in vitro*. This tolerance state is due mainly to the release of ATP through Cx36 hemichannels and activation of purinergic receptors in an autocrine or paracrine manner. P2Y purinergic receptors are known to activate the PLC signal transduction pathway that produces IP_3 and causes the release of calcium from intracellular stores [32], and it was shown in this study that PLC as well as PKA pathway activation is required for the onset of protection induced by depolarization.

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References

[1] M.P. Stenzel-Poore, S.L. Stevens, Z. Xiong, N.S. Lessov, C.A. Harrington, M. Mori, R. Meller, H.L. Rosenzweig, E. Tobar, T.E. Shaw, X. Chu, R.P. Simon, Effect of ischaemic preconditioning on genomic response to cerebral ischaemia: similarity to neuroprotective strategies in hibernation and hypoxia-tolerant states, Lancet 362 (2003) 1028–1037.
[2] K. Matsushima, M.J. Hogan, A.M. Hakim, Cortical spreading depression protects against subsequent focal cerebral ischemia in rats, J. Cereb. Blood Flow Metab. 16 (1996) 221–226.
[3] S. Kobayashi, V.A. Harris, F.A. Welsh, Spreading depression induces tolerance of cortical neurons to ischemia in rat brain, J. Cereb. Blood Flow Metab. 15 (1995) 721–727.
[4] H. Yanamoto, N. Hashimoto, I. Nagata, H. Kikuchi, Infarct tolerance against temporary focal ischemia following spreading depression in rat brain, Brain Res. 784 (1998) 239–249.
[5] A.A.P. Leao, Spreading depression of activity in the cerebral cortex, J. Neurophysiol. 7 (1944) 359–390.
[6] H. Higashida, G. Mitarai, S. Watanabe, A comparative study of membrane potential changes in neurons and neuroglial cells during spreading depression in the rabbit, Brain Res. 65 (1974) 411–425.
[7] E. Sugaya, M. Takato, Y. Noda, Neuronal and glial activity during spreading depression in cerebral cortex of cat, J. Neurophysiol 38 (1975) 822–841.
[8] H. Kager, W.J. Wadman, G.G. Somjen, Simulated seizures and spreading depression in a neuron model incorporating interstitial space and ion concentrations, J. Neurophysiol. 84 (2000) 495–512.
[9] Y. Buresh, V.I. Koroleva, O.S. Korolev, V. Maresh, Changes in the constant potential in brain structures in rats during focal ischemia and systemic hypoxia, Neurosci. Behav. Physiol. 29 (1999) 569–579.
[10] A. Mayevsky, A. Doron, T. Manor, S. Meilin, N. Zarchin, G.E. Ouaknine, Cortical spreading depression recorded from the human brain using a multiparametric monitoring system, Brain Res. 740 (1996) 268–274.
[11] W.D. Dietrich, Z.C. Feng, H. Leistra, B.D. Watson, M. Rosenthal, Photothrombotic infarction triggers multiple episodes of cortical spreading depression in distant brain regions, J. Cereb. Blood Flow Metab. 14 (1994) 20–28.
[12] A.A. Parsons, Cortical spreading depression: its role in migraine pathogenesis and possible therapeutic intervention strategies, Curr. Pain Headache Rep. 8 (2004) 410–416.
[13] G.G. Somjen, Mechanisms of spreading depression and hypoxic spreading depression-like depolarization, Physiol. Rev. 81 (2001) 1065–1096.
[14] H. Zimmermann, Signalling via ATP in the nervous system, Trends Neurosci. 17 (1994) 420–426.
[15] R.D. Fields, B. Stevens, ATP: an extracellular signaling molecule between neurons and glia, Trends Neurosci. 23 (2000) 625–633.
[16] C.G. Dulla, P. Dobelis, T. Pearson, B.G. Frenguelli, K.J. Staley, S.A. Masino, Adenosine and ATP link PCO2 to cortical excitability via pH, Neuron 48 (2005) 1011–1023.
[17] S.C. McKee, C.S. Thompson, L.A. Sabourin, A.M. Hakim, Regulation of expression of early growth response transcription factors in rat primary cortical neurons by extracellular ATP, Brain Res. 1088 (2006) 1–11.
[18] J. Milbrandt, A nerve growth factor-induced gene encodes a possible transcriptional regulatory factor, Science 238 (1987) 797–799.

[19] D. Reigada, C.H. Mitchell, Release of ATP from retinal pigment epithelial cells involves both CFTR and vesicular transport, Am. J. Physiol. Cell Physiol. 288 (2005) C132–C140.
[20] B. Sperlagh, A. Kofalvi, J. Deuchars, L. Atkinson, C.J. Milligan, N.J. Buckley, E.S. Vizi, Involvement of P2X7 receptors in the regulation of neurotransmitter release in the rat hippocampus, J. Neurochem. 81 (2002) 1196–1211.
[21] M.L. Cotrina, J.H. Lin, A. Alves-Rodrigues, S. Liu, J. Li, H. Azmi-Ghadimi, J. Kang, C.C. Naus, M. Nedergaard, Connexins regulate calcium signaling by controlling ATP release, Proc. Natl. Acad. Sci. USA 95 (1998) 15735–15740.
[22] C.E. Stout, J.L. Costantin, C.C. Naus, A.C. Charles, Intercellular calcium signaling in astrocytes via ATP release through connexin hemichannels, J. Biol. Chem. 277 (2002) 10482–10488.
[23] R.A. Pearson, N. Dale, E. Llaudet, P. Mobbs, ATP released via gap junction hemichannels from the pigment epithelium regulates neural retinal progenitor proliferation, Neuron 46 (2005) 731–744.
[24] E. De Vuyst, E. Decrock, L. Cabooter, G.R. Dubyak, C.C. Naus, W.H. Evans, L. Leybaert, Intracellular calcium changes trigger connexin 32 hemichannel opening, EMBO J. 25 (2006) 34–44.
[25] L. Bao, S. Locovei, G. Dahl, Pannexin membrane channels are mechanosensitive conduits for ATP, FEBS Lett. 572 (2004) 65–68.
[26] S.C. Schock, N. Munyao, Y. Yakubchyk, L.A. Sabourin, A.M. Hakim, E.C. Ventureyra, C.S. Thompson, Cortical spreading depression releases ATP into the extracellular space and purinergic receptor activation contributes to the induction of ischemic tolerance, Brain Res. 1168 (2007) 129–138.
[27] R. Schulz, G. Heusch, Connexin43 and ischemic preconditioning, Adv. Cardiol. 42 (2006) 213–227.
[28] C.C. Naus, M.A. Ozog, J.F. Bechberger, T. Nakase, A neuroprotective role for gap junctions, Cell Commun. Adhes. 8 (2001) 325–328.
[29] D.G. Margineanu, H. Klitgaard, The connexin 36 blockers quinine, quinidine and mefloquine inhibit cortical spreading depression in a rat neocortical slice model in vitro, Brain Res. Bull. 71 (2006) 23–28.
[30] A. Wick, W. Wick, J. Waltenberger, M. Weller, J. Dichgans, J.B. Schulz, Neuroprotection by hypoxic preconditioning requires sequential activation of vascular endothelial growth factor receptor and Akt, J. Neurosci. 22 (2002) 6401–6407.
[31] Z. Gajda, Z. Szupera, G. Blazso, M. Szente, Quinine, a blocker of neuronal cx36 channels, suppresses seizure activity in rat neocortex in vivo, Epilepsia 46 (2005) 1581–1591.
[32] M.W. Salter, J.L. Hicks, ATP causes release of intracellular Ca^{2+} via the phospholipase C beta/IP3 pathway in astrocytes from the dorsal spinal cord, J. Neurosci. 15 (1995) 2961–2971.