Published in final edited form as:

Neurochem Res. 2012 June ; 37(6): 1355–1363. doi:10.1007/s11064-012-0720-6.

Pannexin1-Mediated ATP Release Provides Signal Transmission Between Neuro2A Cells

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Abstract

Pannexin1 (Pannx1), a protein related to the gap junction proteins of invertebrates, forms nonjunctional channels that open upon depolarization and in response to mechanical stretch and purinergic receptor stimulation. Importantly, ATP can be released through Pannx1 channels, providing a possible role for these channels in non-vesicular signal transmission. In this study we expressed exogenous human and mouse Pannx1 in the gap junction deficient Neuro2A neuroblastoma cell line and explored the contribution of Pannx1 channels to cell–cell communication as sites of ATP release. Electrophysiological (patch clamp) recordings from Pannx1 transfected Neuro2A cells revealed membrane conductance that increased beyond 0 mV when applying voltage ramps from –60 to +100 mV; threshold was correlated with extracellular K⁺, so that at 10 mM K⁺, channels began to open at –30 mV. Evaluation of cell–cell communication using dual whole cell recordings from cell pairs revealed that activation of Pannx1 current in one cell of the pair induced an inward current in the second cell after a latency of 10–20 s. This paracrine response was amplified by an ATPase inhibitor (ARL67156, 100 μM) and was blocked by the ATP-degrading enzyme apyrase (6.7 U/ml), by the P2 receptor antagonist suramin (50 μM) and by the Pannx1 channel blocker carbenoxolone. These results provide additional evidence that ATP release through Pannx1 channels can mediate nonsynaptic bidirectional intercellular communication. Furthermore, current potentiation by elevated K⁺ provides a mechanism for enhancement of ATP release under pathological conditions.

Keywords

Purinergic receptor; Pannx1; Hemichannel; Neuroblastoma; Intercellular signaling; Paracrine

Introduction

Studies on a wide range of non-excitable cell types and under a wide range of conditions has made it clear that two distinct processes contribute to long range cell signaling: direct intercellular diffusion of second messenger molecules through gap junction channels (most likely IP₃, although Ca²⁺ and other molecules may also contribute) and a nonjunctional pathway in which ATP and other molecules are released from a stimulated cell to act on membrane receptors of adjacent cells [1]. With respect to ATP, its release can elevate Ca²⁺

in adjacent cells through activation of both metabotropic and ionotropic purinergic P2 receptors.

Mechanisms that have been postulated for ATP release from non-excitabile cells include exocytosis, transporters and channels that are permeable to such large anions. Candidate channels include volume sensitive outwardly rectifying anion channels (VSOR), the plasmalemmal form of the mitochondrial voltage dependent anion channel (pIVDAC), gap junction hemichannels (or connexons) in particular those formed of Cx43, and most recently discovered, channels (pannexons) formed of Pannexin1 (Panx1) [2, 3]. Pannexons are mechano- and voltage-sensitive channels that can also be activated following P2 receptor stimulation and by elevated extracellular K^+ ; their activation by these stimuli and their large pore diameter ideally suit them for ATP release [4–7], for which recent evidence has been provided [7–11].

Several studies have begun to characterize the electrophysiological and pharmacological properties of pannexons both in endogenous systems such as the J774 macrophage cell line [5, 12] and primary cultures of astrocytes [13], and when expressed exogenously in *Xenopus* oocytes [6, 7, 14] and in HEK193 cells [15]. We now report results from studies using the Neuro2A neuroblastoma cell line, whose virtual lack of endogenous expression of connexin gap junction proteins has made it the cell line of choice for characterization of biophysical properties of cloned connexins [16]. Neuro2A cells exhibit very low expression of Panx1 [9], allowing evaluation of physiological consequences of exogenous expression [17]. Dual whole cell recordings from Panx1 transfected cell pairs never revealed the presence of high conductance gap junction channels as would be expected if they were formed by this protein. However, these recordings evidenced the contribution of Panx1 channels to non-synaptic paracrine signaling; activation of Panx1 channel opening led to exonucleotidase-sensitive inward currents in an adjacent cell, implicating release of ATP through Panx1 channels from the stimulated cell. Thus, depolarization induced ATP release through Panx1 channels can mediate signaling through activation of ionotropic and metabotropic P2 receptors in this neuroblastoma cell line.

Materials and Methods

Cell Culture

The mouse neuroblastoma cell line Neuro2A originally obtained from American Tissue Type Collection (Rockville, MD, USA) as CCL 131 was used in this study. Cells were cultured in DMEM, supplemented with 10% fetal calf serum (Gibco) and 1% Penicillin/Streptomycin and maintained in a humidified chamber with 5% CO_2 at 37°C. Cells were generally passaged twice weekly and not used beyond twentieth passage.

Electrophysiology

Cells were plated on coverslips for 12–24 h at low confluence prior to recordings. The single or dual whole cell patch clamp configurations [16] were performed at room temperature on cells bathed in external solution containing (mM): NaCl 147, Hepes 10, glucose 13, $CaCl_2$ 2, $MgCl_2$ 1 and KCl 2, pH 7.4. Patch pipettes (resistance 4–6 MOhms) were filled with solution containing (mM): CsCl 130, EGTA 10, Hepes 10, $CaCl_2$ 0.5, and connected to an Axopatch 1D amplifier (Molecular Devices). In some experiments, external K^+ was varied from 0.5 to 10 mM; osmolarity was adjusted by reducing NaCl. Membrane potential was generally held at –60 mV. Voltage activation of Panx1 channels was achieved using voltage ramps from –60 to +100 mV or –60 to +60 mV (16 mV/sec) at 20 s intervals. Data were acquired with Clampex 6.0 or 8.2 software, digitized using an Axon Instruments Digitizer and analyzed with Clampfit 9.0 software (Molecular Devices).

Pharmacological Agents

Drugs used (from Sigma) included tetanus toxin (3 nM, #T3194) to inhibit vesicular release, the gap junction inhibitor carbenoxolone (50 μ M, Sigma), the ectoATPase inhibitor ARL67156 (100 μ M), the ectoATPase apyrase (stock of 200U/ml dH₂O diluted 1:60 in external solution for final concentration of 6.7 U/ml), ATP and BzATP (50 μ M prepared in dH₂O), the non-specific P2R antagonist suramin (50 μ M,) and KN62 (1 μ M), antagonist of the murine P2X₇R.

Transfection with Panx1 cDNA

Neuro2A cells were transfected using 6 μ l oligofectamine reagent (Invitrogen) in 1.5 ml Optimen (Gibco) with 2 μ g of untagged human (h) Panx1 in pCDNA3.1 vector or fluorescent protein (FP) tagged mouse (m) Panx1 in pEYFP or GFP-N1 vectors. The hPanx1 construct was originally obtained from Dr. Gerhard Dahl (U Miami School of Medicine) in a cloning vector; the mPanx1 construct was originally obtained from Dr. Georg Zoidl (Ruhr University, Bochum, Germany) in the pEYFP vector and the mPanx1 sequence was PCR amplified and inserted into the monomeric GFP vector. After overnight exposure, transfection reagents were removed and cells replated on coverslips for an additional 24–36 h incubation in DMEM-FBS medium. Brightly fluorescent cells were chosen for recordings.

Results

Non-synaptic, Panx1-Mediated ATP Release from Neuro2A Cells

Recordings from pairs of Panx1-transfected Neuro2A cells that were either in physical contact or separated from each other by distances up to 50 μ m were used to evaluate whether Panx1 could mediate paracrine signaling to nearby cells. For these experiments, both cells were held at -60 mV and a voltage ramp (-60 to $+100$ mV) was applied to one cell of the pair while continuously recording from both cells (see photographs in Fig. 1d, inset). In 5 of 202 cell pairs that were in physical contact (2.5%), we recorded in the non-pulsed cells small currents (mean conductance: 32 ± 12 pS) that were attributable to the presence of gap junction channels providing a low incidence of a very low degree of electrical coupling between the cell pairs (Fig. 1a, inset). Regardless of whether cells displayed such electrotonic interaction, activation of Panx1 currents (I_{panx}) in one cell by voltage ramps resulted in an inward current in the other cell that began shortly after the end of the voltage ramp and peaked at variable time (~ 20 – 30 s) later (Fig. 1a, b). Using a paradigm in which single voltage ramps were applied to one cell of the pair, we found that 13.7% (7 of 51) of the neighboring cells responded with an inward current. When three identical ramps were successively applied (at 20 s intervals between ramps) to one cell, we found that 19.7% (11 of 56 cells) of the adjacent cells displayed such inward currents (Fig. 1b, c). Such current responses were not seen when the triple ramp protocol was applied to untransfected Neuro2A cells (0/20 pairs, see Suppl. Fig. 1 for example). Neither the peak amplitudes nor total charge transfer differed significantly following single or triple ramp stimulation of Panx1 transfected cells (peak currents 283.2 ± 127.8 vs. 283.2 ± 52.2 pA, total charge 0.15 ± 0.07 vs. 0.16 ± 0.02 μ C; Fig. 1g). Because the percentage of responding cells was $>40\%$ higher using triple than single ramp stimulation, we used this triple ramp paradigm for subsequent experiments.

The presence of a moderate latency (3–20 s) in the response evoked in one cell following voltage ramp stimulation of a neighbor appeared likely to be due to the release of a signaling molecule from the electrically stimulated cell. Consistent with this hypothesis, quantification of the frequency of cells responding with an inward current revealed that such responses were more frequent in cases where cells were close to one another than when distance between cells exceeded 30 μ m, in which case no response was detected (Fig. 1d).

To evaluate whether Ca^{2+} -dependent regulated exocytosis contributes to this paracrine signaling, experiments were performed on cells bathed either in low Ca^{2+} solution or treated with the SNARE degrading tetanus toxin (3 nM). In contrast to what would be expected for a Ca^{2+} -dependent exocytic process, we found that the frequency of responsive cell partners was higher at moderate and low Ca^{2+} concentrations than in the presence of 2 mM extracellular Ca^{2+} (Fig. 1e). Moreover, 20–60 min incubation in zero extracellular Ca^{2+} with 3 nM tetanus toxin did not significantly change the number of cells displaying inward current (25% cells; 8 cell pairs tested). Furthermore, total charge transfer following tetanus toxin treatment was $0.14 \pm 0.09 \mu\text{C}$, similar to that seen with single and triple ramp stimuli (Fig. 1g).

To test whether Panx1 channels were involved in this type of cell–cell communication and to disclose the nature of the molecule(s) released from the electrically stimulated cell, we applied pharmacological agents known to affect Panx1 channels and ATP signaling. As illustrated in Fig. 2a–c, inward current amplitudes (total charge $0.16 \pm 0.02 \mu\text{C}$, peak current $283.2 \pm 52.2 \text{ pA}$; $N = 23$ cells) in response to triple ramps were inhibited by CBX ($0.02 \pm 0.00 \mu\text{C}$ or $32.8 \pm 21.0 \text{ pA}$; $N = 7$ cells), at a concentration that is shown in Fig. 2 to block voltage activated I_{panx1} ($161.2 \pm 19.1 \text{ pA}$, see also Fig. 2f). Moreover, CBX blockade was partially reversible after rinsing (recovering to $0.07 \pm 0.012 \mu\text{C}$ or $234.8 \pm 21.6 \text{ pA}$) (Fig. 2c). Summary of this quantification is shown in Fig. 2g, h.

These results thus indicate that Panx1 is involved in this paracrine signaling. One likely candidate molecule released from the cell through Panx1 channels is ATP, although ATP degradation products such as ADP and adenosine are also active in certain systems and release of other transmitter substances is also possible. In order to evaluate the contribution of ATP, we examined the effects of inhibition or enhancement of ATP degradation in the bathing solution using the ATPase inhibitor ARL67156 (100 μM) or the ATP-degrading enzyme apyrase (6.7 U/ml). For each reagent, we compared the amplitudes of I_{panx1} evoked at +100 mV of the voltage ramp in the stimulated cell before, during and after drug application, as well as the amplitudes and total charges of the inward currents recorded in the nonelectrically stimulated cells. With regard to outward I_{panx1} in the stimulated cell, neither apyrase ($337.8 \pm 32 \text{ pA}$; $N = 7$ cells) nor ARL67156 ($383.0 \pm 28.75 \text{ pA}$; $N = 11$ cells) significantly altered these currents when compared to those recorded from control, untreated cells ($430.6 \pm 49.7 \text{ pA}$; $N = 37$ cells; Fig 2f). With regard to charge transfer and peak amplitudes of inward currents elicited in the cell adjacent to the electrically stimulated one, apyrase caused a large decrease in these parameters (from 0.14 ± 0.03 to $0.017 \pm 0.01 \mu\text{C}$, and from 263.2 ± 22 to $13.6 \pm 9 \text{ pA}$; $N = 5$ cells; Fig. 2g, h), and ARL67156 significantly enhanced these responses (from 0.15 ± 0.02 to $0.29 \pm 0.03 \mu\text{C}$, and from 293.1 ± 27 to $487.5 \pm 106.2 \text{ pA}$; $N = 11$ cell pairs; Fig. 2g, h).

Together, these results are consistent with the hypothesis that opening of Panx1 channels in the depolarized cells leads to the release of ATP that acts through purinergic P2 receptors to activate an inward current in the adjacent cell.

Pharmacological Characterization of Purinergic Receptors in Neuro2A Cells

In order to evaluate which purinergic receptor subtypes were likely to be responsible for the inward current activated by the ATP in Neuro2A cells, we applied P2 receptor agonists and antagonists in voltage clamped cells. Both ATP and the P2X receptor agonist BzATP induced inward currents in Neuro2A cells transfected with mPanx1-GFP (Fig. 3a, b); such biphasic currents are typical of the P2X₇ receptor [18], where the first phase is attributable to the P2X₇ receptor itself and the second to the Panx1 channel opening (see ref. 12). Dose–response curves obtained for ATP and BzATP, where responses were quantified in terms of inward current amplitudes, yielded an EC₅₀ value of 255 μM for ATP and an EC₅₀ value of

64 μM for BzATP; in terms of electrical charge, EC_{50} values were 497 μM for ATP and 82 μM for BzATP (Fig 3c).

Application of suramin (50 μM , a non-specific P2R antagonist) and KN-62 (1 μM , an antagonist of the murine P2X₇R) both reduced ATP-induced inward currents substantially in Neuro2A cells transfected with mPannx1-GFP (after treatment with suramin, electrical charge was reduced from 0.52 ± 0.1 to 0.010 ± 0.02 μC , peak amplitude from 320 ± 39 to 35 ± 9 pA; N = 4 cells; KN62 treatment reduced electrical charge from 0.52 ± 0.2 to 0.01 ± 0.012 μC , peak amplitude from 347 ± 13 to 12 ± 5 ; N = 5 cells (Fig 4a, d and e). Brilliant Blue G (BBG, a rat P2X₇R antagonist) at 100 nM did not totally block ATP induced currents (decreasing electrical charge from 0.42 ± 0.8 to 0.25 ± 0.11 μC and peak current from 335 ± 19 to 135 ± 69 pA; N = 5 cells), even when incubated or perfused for more than 10 min. Inward currents recorded from cells bathed in the presence of ARL67156 induced by triple ramps were decreased by 100nM BBG (from 0.21 ± 0.08 to 0.07 ± 0.05 μC for electrical charge, 238.8 ± 32.2 to 102 ± 52 pA peak current; N = 4 cell pairs), by 50 μM suramin (from 0.21 ± 0.09 to 0.01 ± 0.00 μC for electrical charge, 262.8 ± 11.2 to 22 ± 9 pA for peak current; N = 4 cell pairs; Fig. 4c) and by 1 μM KN62 (from 0.21 ± 0.08 to 0.018 ± 0.02 μC for electrical charge, 302.8 ± 41.2 to 37 ± 12 pA; N = 5 cells pairs; Fig. 4b). ATP induced inward currents during the drug treatment are presented as total electrical charge in Fig. 4d and with respect to electric charge are shown in Fig. 4e. Amplitudes of outward currents evoked in the stimulated cell at 100 mV are quantified before and after each of these treatments in Fig. 4f.

Threshold for Pannexin1 Activation is Reduced when Extracellular K⁺ is Elevated

The strong depolarization used to evoke Pannx1 channel opening may raise the issue of whether this mechanism would be expected to operate under physiological situations. We thus evaluated whether elevating extracellular K⁺, a treatment previously shown by Silverman et al. [11] to facilitate Pannx1 activation, would favor signal transmission and channel opening in our transfectants. As illustrated in Fig. 5a, activation of Pannx1 current occurred at lower voltages as K⁺ level was increased from 0.5 to 10 mM. In Fig. 5b the voltage dependent Pannx1 current in response to 5 s voltage ramps from -60 to $+60$ mV is illustrated. Note that the voltage at which the Pannx1 current is activated is directly related to concentration of potassium in the external solution (threshold -4.6 ± 2.1 mV for 0.5 mM, 3.7 ± 1.1 mV for 2.5 mM, -15.7 ± 0.7 mV for 5 mM and -26.3 ± 0.9 mV for 10 mM K⁺). Figure 5c shows that the fraction of responding cells also increases as concentration of extracellular potassium, is increased. Furthermore, quantification of responses in neighboring cells (Fig. 5d) indicates that the concentration of the amplitude of electric charge transfer in the non stimulated cells also increased at high K⁺.

In order to determine the extent to which amplitude of response in the second cell was dependent on current evoked in the first cell, we correlated values obtained in the experiments where K⁺ was varied. When amplitudes of responses in the second cell were compared to maximal amplitudes of currents in stimulated cells, the relationship was striking, with $r^2 = 0.73$ (Suppl. Fig. 2). This result provides further evidence that a signal carried through Pannx1 channels (likely ATP) mediates the response of the second cell.

Discussion

Whole cell recordings from pairs of Pannx1-transfected Neuro2A cells did not detect formation of inter-cellular channels in Pannx1-transfectants, in contrast to reports in *Xenopus* oocytes and C6 glioma cells [14, 19]. However, currents induced by depolarizing voltages in one cell led to inward currents in the other cell. These currents in the second cell were blocked by treatments that decreased the Pannx1 current in the first cell and were not affected

by blockade of vesicular release (treatment with tetanus toxin or low extracellular Ca^{2+}), indicating that a substance released from the first cell in response to Panx1 channel opening caused a conductance change in the second cell. BBG and suramin had significant blockade on the voltage stimulated current, consistent with recent demonstrations by others that ATP and certain P2 receptor antagonists can block pannexon channels [15, 20].

One likely candidate for the mediating signal is ATP, permeating the Panx1 channels and acting upon purinergic receptors in the adjacent cells. Although metabolites such as ADP, AMP and adenosine might also be active, their major contribution is inconsistent with our findings that blockade of ATPase with ARL67156 potentiated the response and that apyrase reduced it, both of which imply that ATP itself is the main agonist molecule. This was further supported by our finding that the response was blocked by suramin, a broad spectrum P2 receptor antagonist. Agonist dose–response curves and inhibition by selective antagonists demonstrate that P2XRs are main candidates for involvement in this process. However, we have not ruled out minor participation of the G-coupled protein receptors, the P2Y receptors, in the transmission of the ATP signal.

With regard to ATP release, the results obtained in this study indicate that the opening of Panx1 channels by voltage can lead to the release of ATP from the stimulated cell and activation of P2 receptors in adjacent cells. It has long been known that purine and pyrimidine nucleotides are messenger molecules involved in neurotransmission and autocrine/paracrine regulation of cellular functions [21, 22]. Panx1 is widely expressed in most tissues, including both brain and spinal cord, where it is found in both neurons and glia [23–25]. A role for this channel in glial biology likely involves transmission of intercellular calcium waves which are increases in cytosolic Ca^{2+} levels mediated both by direct flux through gap junctions of molecules such as IP_3 and subsequent release of Ca^{2+} from the ER from the neighboring cells and by stimulation of one cell's P2 receptors by extracellular ATP released from the other cell [26, 27]. ATP is released by many cell types through both vesicular mechanisms and ion channels [reviewed in 2]. Although the channel most favored for this role in astrocytes and other cell types has been the Cx43 “hemichannel” [28–31], supporting evidence for this role has primarily consisted of pharmacological blockade by agents that also affect Panx1 channels.

Panx1 expressed in neurons has been proposed as the molecular substrate of “hemichannels” that are activated in ischemic neuronal death [32, 33] and through interaction with the P2X₇ receptor, in oligodendrocyte excitotoxicity as well [34]. The findings that Panx1 is also expressed in postsynaptic density of neurons by electron microscopy [35] and that *N*-methyl-D-aspartate receptors (NMDARs) evoke pannexon currents in the hippocampus [33] suggests that Panx1 is likely to be involved in diverse physiological and pathophysiological nervous system functions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We appreciate the technical support of Ms. Marcia Urban-Maldonado with Panx1 constructs, and the original gifts of hPanx1 and mPnx1 constructs from Dr. Gerhard Dahl (Miami) and Dr. George Zoidl (formerly Bochum, now Toronto). Supported in part by the National Institute of Neurological Disorders (NINDS) of the National Institutes of Health (NIH), NS041282 (to DCS). Studies described here were performed on the Neuro2A cell line to which DCS was introduced by Dr. Robert Ledeen and has been widely used as an exogenous expression system to characterize gap junction channels formed of various connexins (16).

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Fig. 1.

Paired recordings from Pannexin1 transfected Neuro2A cells demonstrate that currents evoked by depolarizing voltage ramps (-60 to $+100$ mV) lead to responses in adjacent cells that are dependent on distance and do not require extracellular Ca^{2+} . **a** Response of a cell to which a voltage ramp was applied (top trace) is shown to be followed by a larger, long lasting inward current in an adjacent cell (lower trace). Note that in some cell pairs a low degree of junctional coupling was present ($g_j = 32 \pm 12$ pS, recorded from 5 cell pairs in which junctional conductance was nonzero), **b** representative traces for a triple ramp protocol. Inward currents were similar to those in the cell pair illustrated in part A following

the ramp applied to the first cell. **c** Quantification of responsive cell for the single and triple ramps protocols with 2 mM Ca^{2+} . **d** Quantification of the percentage of responding cells as a function of distances separating them from the depolarized cell. First bar represents cells likely in direct contact. Note that as distance increased beyond 20 μm , the percentage of responding cells dramatically decreased. Insets show representative photographs acquired for the quantification of the percentage of depolarized Neuro2A cells as a function of distance separating them. **e** Percentages of responsive cells obtained in Neuro2A cell transfected with mPanx1 pEYFP or GFP-N1 at high extracellular Ca^{2+} (2.0 mM), at low extracellular Ca^{2+} (0.5 mM) and in the absence of extracellular Ca^{2+} . Note that cell responsiveness does not depend on extracellular Ca^{2+} concentration and is actually enhanced at low extracellular Ca^{2+} levels. Numbers of voltage clamped cells are in parentheses. **f** Representative traces obtained following tetanus toxin application. **g** Inward currents (quantified as electrical charge) were not significantly different when induced by single or triple ramps applied to the first cell or when recorded in the presence of tetanus toxin. *Numbers in parentheses* represent number of cells for which currents were observed in second cell and analyzed

Fig. 2.

Evidence that ATP released through pannexon channel acts on purinergic receptors in adjacent cells. **a** Response obtained in cell 2 in response to a triplet of ramps applied to cell 1 (control). **b** Inhibition of the depolarization induced current in cell one and inward current evoked in cell 2 by 10 min incubation with 50 μ M CBX. **c** Restoration of currents in cell 1 and cell 2 through rinsing. **d** Amplification of the currents in cell 2 to ramps applied in cell 1 following 10 min incubation with 100 μ M ARL67156. **e** Virtual disappearance of response in the second cell when cells were incubated for 10 min in the presence of apyrase (6.7 U/mL). **f** Quantification of amplitude of outward currents in cell 1 at the peak of the ramp after

exposed to ARL67156 ($P > 0.05$), CBX ($P < 0.05$) and apyrase ($P > 0.05$) and after recovery. Bars represent SEM. Quantification of charge transfer (**g**) and current amplitude (**h**) in cell2 in the presence of CBX, ARL67156 and apyrase. *Numbers in parentheses* represent the number of cells tested

Fig. 3.

Purinergic receptor agonists induce inward currents in Neuro2A cells. **a** Representative traces of inward currents induced by application of 30, 100 and 1000 μM ATP. **b** Representative traces for currents induced by 30, 50 and 100 μM BzATP. Cells were held at -60 mV during agonist application. **c** Dose response current of peak current or total charge recorded in response to application of a range of ATP and BzATP concentrations. EC₅₀ values for ATP and BzATP presented were obtained as best fits to the logistic equation

Fig. 4.

Inward currents recorded in cell 2 are due to activation of purinergic P2 receptors, as identified through use of receptor antagonists **a** Representative traces showing the inhibition of ATP induced inward currents under control condition, after incubation with 1 μM KN62 for 10 min and after rinsing and recovery. Inhibition of the response obtained in cell 2 in response to a triplet of ramps applied to the first cell 10 min after incubation in 1 μM KN62 (**b**) and after treatment with 50 μM suramin (**c**). **d** Quantification of single cell ATP induced currents and of responses in cell 2 to triplet ramp protocol following treatment with 100nM BBG (* $P < 0.05$), 50 μM suramin (** $P < 0.05$) and 1 μM KN62 (** $P < 0.05$) and wash out

($P > 0.05$). **e** Quantification of amplitude of outward currents in cell 1 to BBG ($P > 0.05$), suramin ($P > 0.05$), and Kn62 ($P > 0.05$). *Numbers of recordings are given in parentheses*

Fig. 5.

Evidence that elevated extracellular K^+ facilitates Pannexin1 channel opening. **a** Representative traces showing facilitation of Panx1 currents as concentration of extracellular K^+ is increased. Note that the voltage at which Panx1 current is activated in these ramps from -60 to $+60$ mV is lower at higher K^+ concentration and that maximal current at the end of the ramp is increased at higher K^+ . **b** Quantification of currents in 10 mV increments from -60 to 60 mV obtained from voltage ramps in the presence of K^+ concentrations 0.5 ($n = 31$), 2.5 ($n = 7$), 5 ($n = 16$), and 10 mM KCl ($n = 6$). Asterisk indicates $P < 0.05$. **c**, **d** Increase in number of responding cells and amplitude of responses as K^+ concentrations were increased from 2.5 to 10 mM