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Intracellular Signaling Pathways Integrating the Pore Associated with P2X7R Receptor with Other Large Pores

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1. Introduction

The purinergic $P2X_7$ receptor (P2X7R) is a member of the family of ligand-gated ion channels composed of seven subtypes, $P2X_{1-7}$. These receptors possess three subunits assembled as homo- or heterotrimers to make functional receptors (Nicke et al., 1998, 2005), which has been confirmed by atomic force microscopy (Barrera et al, 2005) and by crystallography (Kawate et al, 2009). The P2X7R shares an overall membrane topology with the other members of this family of receptors; it contains two putative pore forming transmembrane segments, a large cysteine-rich ligand-binding extracellular domain, and intracellularly located N and C termini (Surprenant et al, 1996). This subtype is structurally distinguished from other members of P2XRs by its long intracellular C-terminal tail with multiple protein and lipid interaction motifs, besides a cysteine-rich 18 – amino acid segment. From a pharmacological point of view, the P2X7R requires at least a 100-fold higher ATP concentration for activation compared to other P2XRs (North, 2002). Extracellular divalent cation reduction increases agonist potency (Hibell et al, 2001; Michel et al, 1999). Moreover, extracellular cations and chloride have important effects on the channel gating (Gudipaty et al., 2001; Li et al., 2003, 2008, 2010; Riedel et al., 2007b; Virginio et al., 1997). In this context, P2X7R activation can sustainably induce a wide range of different intracellular signaling responses (Dubyak, 2007; North, 2002).

P2X7R, when activated by ATP or the potent agonist 3'-O-(4-benzoyl)benzoyl-ATP (BzATP), functions as a non-selective cation channel, permeant to small cations, such as Na^+, K^+, and Ca^{2+} and this activation mode is dependent on extracellular divalent cations (Ding & Sachs, 2000; Jiang, 2009; Ma et al, 2006; Sperlágh et al, 2006; Virginio et al, 1997). Upon repeated or prolonged application of agonist, the P2X7R becomes permeable to larger molecules like ethidium bromide, N-methyl-D-glucamine or neurotransmitters such as glutamate and ATP (Faria et al, 2005, 2010; Hamilton et al, 2008; Jiang et al, 2005; Marcoli et al, 2008), a process termed cell "permeabilization" or large conductance channel opening.

Up to now, several ideas have been proposed as a possible explanation to this pore opening (Alloisio et al, 2010; Coutinho-silva et al, 1997; Faria et al, 2005, 2009; Jiang et al, 2005; North,

2002; Pelegrin & Surprenant, 2006; Virginio et al, 1999; Yan et al, 2008). In a general manner, cell permeabilization can be observed in cell types transfected or natively expressing P2X7R, in contrast to Xenopus oocytes transfected cells (Petrou et al, 1997) or lymphocytes B cells of patients with Chronic lymphocytic leukaemia (Boldt et al, 2003; Gu et al, 2000).

Initially, some groups have proposed that the opening of the pore associated to P2X7R receptor occurs after the small channel (8 pS) allosterically changes and expands over time-dilatation (Chaumont & Khakh, 2008; Virginio et al, 1999; Yan et al, 2008). Others have reported that Pannexin-1 (panx-1), a hemichannel protein, is the large conductance channel that opens independently of the cationic P2X7R (Locovei et al, 2007; Pelegrin & Surprenant, 2006, 2007). On the other hand, some groups have demonstrated another putative explanation of the dye uptake. They have reported two possible charge selectively pathways that are activated by P2X7R: one for cationic and another for anionic dyes (Cankurtaran-Sayar et al, 2009; Schachter et al, 2008). Jiang and collaborators have proposed distinct pathways to permeate inorganic monovalent and divalent cations, organic cations ($NMDG^+$) and fluorescent dyes (Jiang et al, 2005). In addition, it has been suggested at least two different conductive pathways, one for Ca^{2+} and other for monovalent ions (Alloisio et al, 2010).

In this context, some groups have published that the P2X7R permeabilization depends on intracellular factors (Donnely-Roberts et al, 2004; Faria et al, 2005, 2010; Gu et al, 2009; Le Stunff & Raymond, 2007; Shemon et al, 2004; Zhao et al, 2007) to occur.

In this chapter, we come up with novel proposals of intracellular signaling regulation that would help us to understand about the several intriguing characteristics of the ATP-induced P2X7R.

2. Intracellular signalling associated with small conductance channel

In the P2X7R receptor gating mechanism (low conductance channel) the response to agonist challenge, allow rapid, non-selective passage of cations across the cell membrane. It is permeable to Na^+ and K^+ and presents high permeability to Ca^{2+} (North, 2002). The cellular response to P2X7R receptor activation after agonist exposition is generally very rapid and this reaction does not depend on the production and diffusion of second messengers within the cytosol (Burnstock, 2007; Ralevic & Burnstock, 1998). In contrast, an increase in the intracellular Ca^{2+} concentration and a consequent depolarization of the cell membrane are observed after the ionic channel opening, subsequently activating voltage-gated calcium channels. In addition, evidence indicates that the large Ca^{2+} ion concentration in the cytoplasm could activate intracellular kinases like protein kinase C (PKC), mitogen activated protein kinases (MAPKs), calcium–calmodulin-dependent protein kinase II (CaMKII) (Amstrup & Novak, 2003; Bradford & Soltoff, 2002; Heo & Han, 2006), caspases (Orinska et al, 2011), phosphoinositide 3-kinase (Pi3K) (Jaques-Silva et al, 2004) and phospholipases (Alzola et al, 1998; Perez-Andres et al, 2002; Pochet et al, 2007). Further, other functions may be mediated by P2X7R receptor activation such as IL-1β maturation (Ferrari et al, 1997), shedding of membrane proteins (Moon et al, 2006); Src (Denlinger et al, 2001), and glycogen synthase kinase 3 (Ortega et al, 2009); membrane blebbing (Morelli et al, 2003).

These different signaling pathways may contribute to a large complexity in the response of this receptor and it raises the question about how the correct coupling and fine tuning of the signaling in response to extracellular stimuli is achieved. To date, several pieces of evidence

have been described about the small conductance P2X7R receptor and interactions with other proteins (Adinolfi et al, 2003; Antonio et al, 2011; Barbieri et al, 2008; Boumechache et al, 2009; Bradley et al, 2010; Denlinger et al, 2001; Guo et al, 2007; Lemaire et al, 2007; Liu et al, 2011; Wilson et al, 2002), mainly through its longer C-terminus region or lipids (Denlinger et al, 2001; Gonnord et al, 2009; Michel& Fonfria, 2007; Takenouchi et al, 2007; Zhao et al, 2007a, 2007b). However, there are only a few studies about the low conductance P2X7R receptor gating and the mechanism of transition to large conductance channel opening. This may be due to (i) a lack of selective agonists or antagonists only to small or large channel (North, 2002) or (ii) to P2X7R receptor polymorphisms or (iii) to artificial deletions in regions of this receptor resulting in distinct intracellular or extracellular regulation of this phenomenon. In this context, the concept that the C-terminal domain of P2X7R directly regulates a complex distinct from receptor-dependent pore activity was first introduced by El-Moatassim and Dubyak (El-Moatassim & Dubyak, 1992). They demonstrated that P2X7R receptor mediated phospholipase D (PLD) activity was dependent on GTP and independent of the large conductance channel opening. Another study found that human $P2X_7$ receptor currents were facilitated in response to repeated or prolonged agonist applications, via dynamic calmodulin binding (Roger et al, 2008). This Ca^{2+}-dependent component is related to the uptake of large compounds seen by the pore complex. These and other papers (Alloysio et al, 2010; Boldt et al, 2003; Le Stunff et al, 2004) suggest independent intracellular signaling pathways regulating the low and large conductance channel associated with P2X7R .

3. Intracellular signalling pathways associated with large conductance channel

The intracellular regulation of the P2X7R associated large conductance channel opening is still mostly unknown. The initial suggestions for dependency of cytoplasmic factors in this event were originated from electrophysiological data in outside or inside out configurations with no large conductance channel recordings (Coutinho-Silva & Persechini, 1997; Persechini et al, 1998; Petrou et al, 1997). Posteriorly, other groups have investigated the P2X7R pore formation induced by intracellular signaling and how mutated amino acids or truncated regions affect functional availability of the receptor. In this line, Smart used truncated and single-residue-mutated P2X7R receptors in HEK-293 cells and in Xenopus oocytes. Truncated P2X7R at residue 581 (of 595) were not able to dye uptake, but there was dye uptake similarly to the wild receptor in those cells expressing the truncated P2X7R at position 582. In contrast, the small channel function was only suppressed in the residues 380 (Smart et al, 2003). Two alternative splices variants were identified in the human P2X7R (one lacking the first transmembrane domain and the other the entire cytoplasmic tail, but they were compared to the full-length channel). The first variant exhibited a non-functional slow conductance channel, while the second did not affect the small ion channel activity, but affected the large conductance channel and caspase activation (Cheewatrakoolpong et al, 2005). In addition, threonine 283 (Thr283) has been described as a critical residue in the ectodomain for P2X7R receptor function and it has been suggested that the intracellular leucine residue (P451L) alters downstream signalling independently of ion channel activity (Young et al, 2006). Recently, Marques-da-Silva and collaborators (Marques-da-Silva et al, 2011) demonstrated that colchicine did not inhibit ATP-evoked currents in macrophages, but it decreased ATP-induced dye uptake. Large conductance channel opening on Xenopus

oocytes and HEK293 cells expressing P2X7R were inhibited after colchicines treatment (Marques-da-Silva et al, 2011). Yan described that extracellular Ca^{2+} concentration is a physiological negative modulator of the P2X7R low conductance channel without affecting the large conductance channel opening (Yan et al, 2011).

There is some controversial data related to the biophysical, pharmacological or molecular tools that impair the actions of intracellular enzymes involved in the P2X7R pore formation. In one of the primary papers studying the intracellular signaling of the P2X7R large conductance channel, it was described that calmidazoliun, an inhibitor of the calmodulin protein, impaired the small channel activity, but had no effect in the large conductance channel opening (Virginio et al, 1997). This result was also confirmed by other groups (Donnelly-Roberts et al, 2004; Faria et al, 2010; Lundy et al, 2004). However, Roger and coworkers (Roger et al, 2008, 2010) reported that rat P2X7R induced large organic cation permeability ionic currents were dependent on critical residues of calmodulin binding domain when recorded in patch-clamp whole cell configuration. In this sense, the intracellular Ca^{2+} concentration dependence in the P2X7R pore formation is still unclear. We have shown in 2005 that intracellular Ca^{2+} acts as a second messenger in the large conductance channel opening in peritoneal macrophages and 2BH4 cells (Faria et al, 2005). Similar data were found by other groups (Cankurtaran-Sayar et al, 2009; Roger et al, 2008, 2010; Schachter et al, 2008), but others did not observe this effect (da Cruz et al, 2006; Iglesias et al, 2008; Schachter et al, 2008; Virginio et al, 1999). We continued to investigate the Ca^{2+} participation in more detail, and we described a major Ca^{2+} dependence in the P2X7R pore formation, but we also observed Ca^{2+} independent events in the same cell types (Faria et al, 2010). This variability in the responses may be due to preponderant expression of distinct P2X7R variants or activity of different large conductance channels, as was discussed above in the text. Phospholipase C (PLC) had no effect on P2X7R large conductance channel formation in THP-1 cells (Donnelly-Roberts et al, 2004), or in mouse 2BH4 cells or peritoneal macrophages cells (Faria et al, 2010). However, P2X7R pore formation was inhibited in mouse microglial cell line by PLC (Takenouchi et al, 2005). It was also shown that MAPK is associated with P2X7R activation (Donnelly-Roberts et al, 2004; Faria et al, 2005, 2010), but in other hands this was not confirmed (da Cruz et al, 2006; Michel et al, 2006). These discrepancies may be due to species variations, distinct intracellular machinery or differences in the protocol used to investigate a specific function in a same cell type (Faria et al, 2010). Other proteins have presented less divergent responses, such as: PKC (Donnelly-Roberts et al, 2004; Faria et al, 2010; Shemon et al, 2004), Ca2+-insensitive Phospholipase A PLA (Chaib et al, 2000), caspase-1 and-3 (Donnelly-Roberts et al, 2004; Faria et al, 2010), PLD (Stunff & Raymond, 2007), phosphatidylinositol 4,5-bisphosphate (PIP_2) (Zhao et al, 2007), cytoskeleton components (Marques-da-Silva et al, 2011), PI3K (Faria et al, 2010), src tyrosine phosphorylation (Iglesias et al, 2008), Peroxisome proliferator-activated receptor gamma (PPAR gamma) (Nagasawa et al, 2009a) antagonists and intracellular Ca^{2+} chelants (Faria et al, 2005, 2010).

As mentioned above, one possible drawback in relation to these results is due to the diversity of responses observed in $P2X_7$ species and cell types (Donnelly-Roberts et al, 2009; Michel et al, 2008). The variations may be proportional to natural P2X7R receptor polimorphisms and these may be, at least partially, functional promoting gain or loss of activity (Cheewatrakoolpong et al, 2005; Feng et al, 2006; Masin et al, 2011; Shemon et al, 2006). Another related factor to this matter is the native structural state of the P2X7R

receptor. In this line, a monoclonal antibody (Ab) to P2X7R ectodomain was used to immunoprecipitate the receptor complex in central and peripheral immune cells (Kim et al, 2001b). Using western blotting, native P2X7R in peritoneal macrophage or bone marrow cells formed bound multimeric complex with numerous bands ranging in size from 25 up to 250 kDa, in contrast to P2X7R from brain glia and/or astrocytes that formed only monomeric subunits. This result suggests differential intracellular regulation of the P2X7R pore in distinct cell types (Kim et al, 2001b). Li and coworkers discovered in parotid acinar and duct cells a cell-specific assembly and gating of the P2X7R channels, in a way that upon exposure to ATP, P2X7Rs are assembled into functional channels with rapid gating. In contrast, P2X7Rs from duct cells are preassembled and continually subject to rapid gating by ATP (Li et al, 2003). Recently, other researches have found distinct pathways of dye uptake, mediated by P2X7R receptor activation after ATP treatment, possibly through different large conductance channels. Schachter and collaborators compared P2X7R-associated cation and anionic fluorescent dyes uptake of macrophages and HEK-293 cells transfected with P2X7R receptor (Schachter et al, 2008). Transfected cells did not take up anionic dyes and did not display single channel cell-attached recordings, in contrast to the native mice peritoneal macrophages. Anionic and cationic dye effluxes induced by ATP treatment were temperature independent and dependent, respectively (Schachter et al, 2008). In addition, another study examined the process of dye uptake by transfected or natively expressed P2X7R receptor leading to the pore formation. HEK-293 cells expressing rat P2X7R was permeable to cationic but not to anionic dyes in a way that intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase was not necessary to be activated (via 1). In the via 2, the pore was permeated only by lucifer yellow and it was completely dependent on $[Ca^{2+}]_i$ for activation. Also, RAW 264.7 cells presented both pathways similar to the transfected cells, but they did not require intracellular Ca^{2+} (Cankurtaran-Sayar et al, 2009).

Based on all these data from different groups suggesting that more than one pore might work simultaneously after ATP treatment, we describe the intracellular enzymes activated by the P2X7R associated large conductance channel opening compared to other large conductance channels.

4. Intracellular signaling cascades activated by these pores

Since the proteins responsible to the P2X7R large conductance channel opening are still largely unknown here we compare and discuss the intracellular signaling pathways and the possible candidates associated to the P2X7R pore formation. Among them are connexin hemichannels, pannexin-1, plasma membrane voltage dependent anion channel (pl-VDAC), maxi anion, transient receptor potential vaniloid-1 (TRPV1), transient receptor potential anquirin-1 (TRPA1), Maitotoxin-induced pore and Rising of intracellular Ca^{2+} concentration induced pore.

The connexin hemichannel, a hexameric protein composed of connexin subunits expressed in vertebrates, was the first large conductance channel studied with functional similarity to P2X7R receptor large conductance channels. An initial study used two types of J774 mouse macrophages, one sensitive and another ATP-insensitive. In the sensitive cells, connexin-43 (Cx43) gap junction mRNA and protein and P2X7R were expressed and the dye was taken up, but in the insensitive lineage there was not Cx43 expression and neither dye uptake. Therefore, they proposed that connexin 43 was the pore associated to P2X7R receptor (Beyer

& Steinberg, 1991). This concept was elegantly refuted by Alves and colleagues at least in peritoneal macrophages where they demonstrated that experimental conditions known to block hemichannels and Cx43 knockout mice maintained the P2X7R large conductance channel activity (Alves et al, 1996). Also, P2X7R and Cx43 are expressed in J774 macrophage lineage and are colocalized in the cell membrane (Fortes et al, 2004).

In relation to the intracellular signaling cascades, connexins can be modulated in the C-terminal domain by phosphorylation through PKC (Bao et al, 2007; Hawat & Baroudi, 2008), MAPK (Bao et al, 2007), S-nytrosylation with covalent biding of nitric oxide (NO) to cysteine (Cys) (De Vuyst et al, 2007; Retamal et al, 2009), protein kinase A (Liu et al, 2011), intracellular redox potential (Retamal et al, 2007) and intracellular Ca^{2+} concentration (De Vuyst et al, 2006; Schalper et al, 2008; Thimm et al, 2005). Compared to the P2X7R receptor pore, the connexin hemichannel may be dependent on intracellular Ca^{2+}, PKC and MAPK. Meanwhile, the unitary conductance value (20-250pS) for all known connexins are lower compared to the ones observed to P2X7R receptor (400pS). In addition, it has been shown that connexin hemichannel blockers have no effect on the P2X7R receptor pore formation (Faria et al, 2005) and this apparently ruled out the participation of connexins at least in cell types tested.

Maitotoxin (MTX), a marine toxin, described to increase calcium in GH4C1 rat pituitary cells (Young et al, 1995), increases intracellular Ca^{2+} concentration leading to the opening of a pore with biophysical properties similar to P2X7R large conductance channel (Schilling et al, 1999a, 1999b). The dye uptake observed after this pore opening may also be dependent on extracellular Ca^{2+} (Lundy et al, 2004; Wisnoskey et al, 2004), intracellular Ca^{2+} concentration (Wisnoskey et al, 2004), calmodulin (Donnelly-Roberts et al, 2004; Lundy et al, 2004) and PLC (Donnelly-Roberts et al, 2004). Although the maitotoxin pore may be functionally similar to the P2X7R pore, they might possess different intracellular pathways. A possible explanation to this fact may be that the same large conductance channel or different similar pores functioning in conjunction might be regulated by distinct signaling pathways

In 2009, our group described a large conductance channel stimulated by rising of intracellular Ca^{2+} concentration recorded in cell attached patches. This pore was blocked by calmodulin, Calcium-calmodulin kinase type II (CamKII), PLC, MAPK and caspase-1 and-3 antagonists and it was insensitive to PKC and P2X7R receptor antagonists (Faria et al, 2009). The intracellular signaling pathways modulating this pore and the one associated with P2X7R are distinct, but they possessed some common pathways. In addition, the pore induced by MTX presents large intracellular signalling similar to the pore described by us. Since the protein responsible for the opening of the MTX induced pore also is not indentified, the pore recorded in our conditions (Faria et al, 2009) may be the same as the MTX pore.

Another large conductance channel, that may be activated by rising of intracellular Ca^{2+} concentration (Locovei et al, 2006; Ma et al, 2006), is the pannexin hemichannel, which is a hexameric protein present in vertebrates and invertebrates. However, as reported by recent papers, the extracellular or intracellular Ca^{2+} did not interfere with the pannexin activity (Ma et al, 2009; Pelegrin & Surprenant, 2007). This large conductance channel might be activated by S-nitrosylation and Src kinase (Iglesias et al, 2009; Pelegrin & Surprenant, 2006; Suadicani et al, 2009). P2X7R receptor large conductance channel was inhibited by RNAi to pannexin-1, inhibitory peptide and pannexin antagonists (Pelegrin & Surprenant, 2006). In contrast, other groups did not observe inhibition of the P2X7R large conductance channel

for pannexin-1 inhibitors or RNAi (Faria et al, 2005, 2010; Nagasawa et al, 2009b; Reyes et al, 2008; Schchater et al, 2008; Yan et al, 2008, 2011). Up to now, for some groups the pannexin-1 seems to be an important player in this phenomenon, but apparently this protein is working in conjunction with other protein(s). This information is based on the partial blockage of the P2X7R receptor induced dye uptake exhibited after pannexin-1 inhibition (Iglesias et al, 2009; Locovei et al, 2007; Pelegrin & Surprenant, 2007). Alternatively, other large conductance channel such as MTX-induced pore (Pelegrin & Surprenant, 2007), P2X2R large conductance channel (Marques-da-Silva et al, 2011) and the rising of intracellular Ca^{2+} induced pore (Faria et al, 2009) were not impaired by pannexin-1 inhibitors.

Maxi-anion channel possesses a wide nanoscopic pore suitable for nucleotide transport and an ATP-binding site in the middle of the pore lumen to facilitate the passage of the nucleotide (Sabirov & Okada, 2004). Physiologically, the same large conductance channel is operational in swelling-, ischemia-, and hypoxia-induced ATP release from neonatal rat cardiomyocytes (Dutta et al, 2004). In addition, raising the intracellular Ca^{2+} concentration (Bajnath et al, 1993; Groschner & Kukovetz, 1992; Hussy, 1992; Kawahara & Tawuka, 1991) as well as protein tyrosine dephosphorylation (Toychiev et al, 2009) can activate this pore. On the other hand, PKA (Okada et al, 1997), PKC (Kokubun et al, 1991; Saiguza & Kokubun, 1988; Vaca & Kunze, 1993), G proteins (Schwiebert et al, 1992; Sun et al, 1993) and Src kinase (Kajita et al, 1995) antagonists can inhibit it. This large conductance channel also has no protein constituents identified so far (Sabirov & Okada et al, 2009) and there are no studies comparing its effects with other pores, except to pl-VDAC (Sabirov et al, 2006).

Voltage-dependent anion channels (VDACs) were originally characterized as mitochondrial porins but other evidence began to accumulate that VDACs could also be expressed in the plasma membrane (pl-VDAC). VDAC may be activated changing the applied voltage in the presence of NADH (Zizi et al, 1994) and under apoptotic conditions (Elinder et al, 2005). In relation to the intracellular signaling pathways, there are few data up to now, but some groups have shown the involvement of this pore with lipid rafts (Ferrer, 2009; Herrera et al, 2011). Moreover, the large conductance channel and pl-VDAC may be activated by excised patch, indicating an independence of the intracellular signals to open the large conductance channel (Guibert et al, 1998; Sun et al,1993). Relevant information is about the nucleotide-binding sites in the C-terminus of the mitochondrial VDAC, which presents the same C-terminal sequence of the pl-VDAC (Yehezkel et al, 2006). The main discrepancy in relation to both pores compared to the P2X7R pore is due to the lack of single channel recordings of the P2X7R pore in excised patches (Riedel et al, 2007). This fact may indicate that maxi anion and pl-VDAC pores are different compared to the P2X7R receptor pore, since they did not depend on cytoplasmic factors to open. But, this does not rule out the participation of this pore in this phenomenon.

The capsaicin induced receptor, transient receptor potential vanilloid 1 (TRPV1), is activated not only by capsaicin but also by heat (>43^{o}C), acid and various lipids (Moran et al, 2011). Since capsaicin and its analogues, such as resiniferatoxin (RTX), are lipophilic, it is quite possible that they pass through the cell membrane and act on the binding sites present in the intracellular surface of TRPV1. It is a Ca^{2+} permeable non-specific cation channel. It was demonstrated that activation of native or recombinant rat TRPV1 leads to time- and agonist concentration-dependent increase in the relative permeability of large cations and changes in Ca^{2+} permeability (Chung et al, 2008). TRPV1 induced small channel can be modulated by calmodulin, PKC, PKA, intracellular Ca^{2+}, PLC, G protein and PIP_2/ Src (Bhave et al, 2002;

Chuang et al, 2001; Dai et al, 2004; Moriyama et al, 2003, 2005; Sugiura et al, 2002; Tominaga et al, 2001). TRPV1 induced a large conductance channel similar to the P2X7R since it depends on the C-terminus and it is modulated by PKC phosphorylation (Chung et al, 2008). Although biophysically this pore is similar to the P2X7R associated pore, the intracellular signaling is poorly understood up to now.

TRPA1 is a nonselective cation channel that belongs to the superfamily of mammalian TRP ion channels and is unique since it possesses a large number of ankyrin repeats in its N-terminal domain (Montell, 2005). TRPA1, when activated, are permeable to small cations such as Ca^{2+}, K^{+}, Na^{+}; simultaneously it depolarizes the plasma membrane and raises intracellular Ca^{2+}, which subsequently triggers a variety of physiological responses. Recently, it was described that TRPA1 activation induces dye uptake, which is blocked by selective TRPA1 antagonists. In addition, outside-out patch recordings using N-methyl-D-glucamine ($NMDG^{+}$) as the sole external cation and Na^{+} as the internal cation, TRPA1

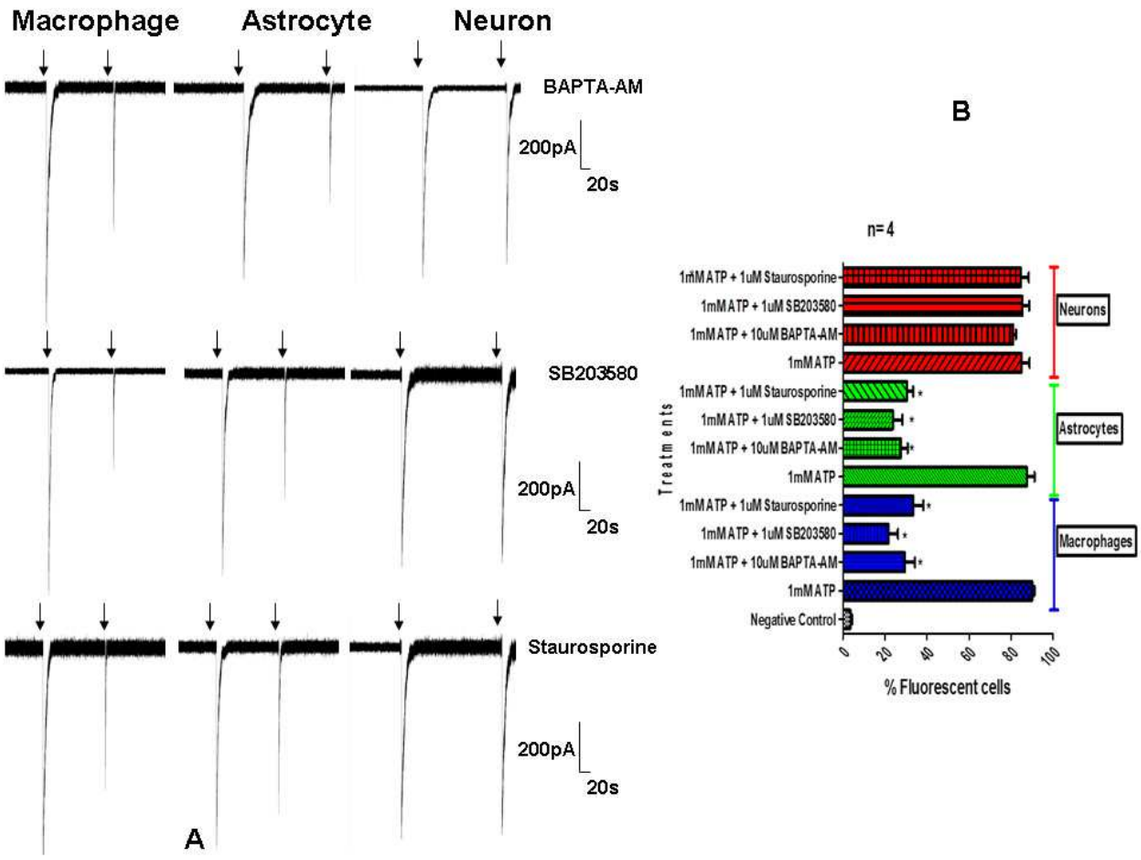

Fig. 1. Pharmacological comparison of the intracellular pathway of the pore associated with $P2X_7$ receptor in different cell types. A- Whole cell experiments in mice peritoneal macrophages, mice cortical astrocytes or mice mesencephalic. We applied 1mM ATP, after the incubation of the cells with 10μM BAPTA-AM, 1μM Sb203580 or 1μM Staurosporine for 5 minutes at 37°C. B- The graphic represents the quantification of dye uptake experiments in the cell types cited above. The values represent the mean ± SD of three to four experiments performed on different days. *$p<0.05$ compared with the ATP treatment of each group.

activation results in dynamic changes in permeability to $NMDG^+$ (Chen et al, 2008). Other groups have reproduced this data (Banke et al, 2010, 2011), but in every cell studied the intracellular signaling was not investigated. Moreover, the fact that TRPA1 associated large conductance channel permeates large cations in outside out configuration suggests a possible independence of intracellular factors.

As we can observe above, there are diverse common intracellular signaling proteins that may be used in the activation of these large conductance pores. Moreover, these pores might be biophysically and functionally similar. Thus, when we performed assays which preincubated cells with intracellular signaling pathway blockers were stimulated with 1mM ATP, there was an activation of the large conductance channel associated to P2X7R (Figure 1), as previously shown in our published papers (Faria et al, 2005, 2010). Using whole cell configuration, we used (i) neurons to evaluate the P2X7R pore formation in cells expressing TRPV1 and TRPA1; (ii) astrocytes to study the expression of Maxi anion, pl-VDAC, Connexin 43 and Pannexin-1; and (iii) macrophages to study Maitotoxin and intracellular Ca^{2+} increase induced pores.

5. Conclusions

Finally, based on data discussed here, several issues might explain why a common gate mechanism for the P2X7R pore is not yet understood: (1) a large conductance channel is activated by different signaling pathways; (2) these signaling cascades might be related to the activation of distinct pores; (3) both [(1) and (2)] mechanisms might act together in certain cells; (4) it might exist a gate modulator that is cell-type specific, (5) $P2X_7$ might be part of a macromolecular protein complex or a protein-lipid complex. In summary, more studies are necessary in order to comprehend the functional mechanism of the $P2X_7$ receptor.

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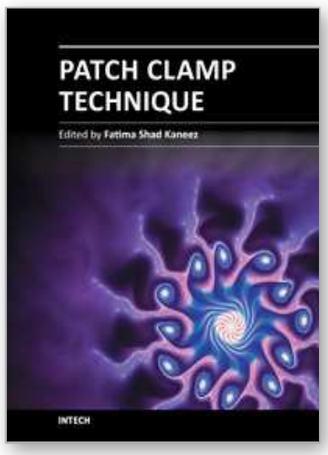

Patch Clamp Technique
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This book is a stimulating and interesting addition to the collected works on Patch clamp technique. Patch Clamping is an electrophysiological technique, which measures the electric current generated by a living cell, due to the movement of ions through the protein channels present in the cell membrane. The technique was developed by two German scientists, Erwin Neher and Bert Sakmann, who received the Nobel Prize in 1991 in Physiology for this innovative work. Patch clamp technique is used for measuring drug effect against a series of diseases and to find out the mechanism of diseases in animals and plants. It is also most useful in finding out the structure function activities of compounds and drugs, and most leading pharmaceutical companies used this technique for their drugs before bringing them for clinical trial. This book deals with the understanding of endogenous mechanisms of cells and their receptors as well as advantages of using this technique. It covers the basic principles and preparation types and also deals with the latest developments in the traditional patch clamp technique. Some chapters in this book take the technique to a next level of modulation and novel approach. This book will be of good value for students of physiology, neuroscience, cell biology and biophysics.

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