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REVIEW

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Regulation of TRPM2 channels in neutrophil granulocytes by ADP-ribose: a promising pharmacological target

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Abstract TRPM2 channels play an important role in the activation process of neutrophil granulocytes. One mechanism of TRPM2 channel gating is the binding of intracellular ADP ribose (ADPR) to the Nudix box domain in the C-terminal tail of TRPM2. Intracellular Ca^{2+}, although not an activator of TRPM2 by its own, significantly enhances TRPM2 gating by ADPR. Stimulation of neutrophil granulocytes with the chemoattractant peptide *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) induces release of Ca^{2+} ions from intracellular stores which in cooperation with endogenous ADPR levels enable Ca^{2+} influx through TRPM2. Stimulation of the ectoenzyme CD38, a membrane-associated glycohydrolase with ADPR as main product, and uptake of ADPR into the cell may contribute to the effects of fMLP. Inhibition of ADPR production, of uptake and of binding to TRPM2 are all potential pharmacological principles by which a modulation of neutrophil function may become possible in future.

Keywords Calcium influx · Channel blocker · Hydrogen peroxide · ADP-ribose · Chemotaxis · NAD · CD38 · Glycohydrolase

Block of Ca^{2+} channels: a pharmacological principle not yet realised for the TRP family

Inhibition of calcium entry is one of the most successful pharmacological principles. Prominent examples are the voltage-dependent L-type Ca^{2+} channels in vascular smooth muscle and heart muscle cells (McDonald et al. 1994; Perez-Reyes and Schneider 1995; Hofmann et al. 1999), targeted by several classes of drugs such as the dihydropyridines. These substances have been used now for decades mostly as vasodilators (Godfraind 1994), although their potential and practical pharmacological spectrum reaches far beyond (van Zwieten 1998; Snutch et al. 2001; Romero et al. 2003).

L-type channels are typically expressed in electrically excitable cells where they are activated by depolarisation of the membrane potential. In electrically non-excitable cells, other mechanisms are employed to induce Ca^{2+} influx, and Ca^{2+} channels distinctly different from classical voltage-dependent channels constitute Ca^{2+} entry pathways. The importance of Ca^{2+} influx, however, is by no means less outstanding than in excitable cells. There are two features of Ca^{2+} influx in non-excitable cells that make it a promising target for future pharmaceutical developments (Li et al. 2002b). First, increases in the cytosolic Ca^{2+} concentration evoked by Ca^{2+} influx represent an almost universal signal for cell activation. This activation may initiate cell functions as different as chemotaxis in leukocytes, aggregation in platelets or release of bioactive factors in endothelial or secretory epithelial cells, among many other examples, but nearly all of them may become pathophysiologically relevant mechanisms with key roles in some specific pathological conditions. Therefore, the inhibition of such functions might offer extreme therapeutical benefits. Second, although Ca^{2+} influx is a universal signal and many cells use common principles in their regulation of Ca^{2+} influx, evidence is now emerging that on a molecular basis, there exists an ample variety and specificity of Ca^{2+} influx between cell types. Such cell-specific peculiarities of Ca^{2+} entry regulation may potentially be used for the development of drugs that exclusively target distinct types of cells or may even discriminate within the same cell type, on the basis of the functional state of the cells.

Up to now, however, there is hardly any pharmacological principle in use that would involve a direct interaction with Ca^{2+} influx in non-excitable cells, i.e. blockade of voltage-independent channels. Efforts into this direction have only recently been reported, e.g. by Li et al. (2005). The surprising delay in the realisation of these channels as

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pharmacological targets is mostly due to the fact that the molecular mechanisms underlying Ca^{2+} influx were extremely poorly understood for a long time, in spite of all efforts of researchers of the field. Until very recently, the molecules that participate in the formation of Ca^{2+} entry channels were completely unknown, as were the exact mechanisms responsible for their regulation. The turning point was the discovery of human homologues of the *Drosophila* trp gene in the mid 1990s. The human TRP family includes several subfamilies (Clapham et al. 2003); when only the subfamilies TRPC, TRPM and TRPV are considered, about 25 genes have been identified that code for Ca^{2+}-permeable channels or subunits of such channels. Channels that result from the heterologous expression of particular TRPs can be studied in detail with respect to their permeation properties and, more importantly, their mode of regulation. Heterologous expression models would also allow screening tests for the development of specific blockers. However, even though our molecular knowledge of voltage-independent cation channels has exploded over the last decade, our level of understanding is still far less than satisfactory. Major questions relate to which TRP molecules (in form of either homomultimers or heteromultimers) participate in the formation of Ca^{2+} entry pathways as they are expressed in native cells (in distinction to TRP expression models), how exactly these pathways are regulated and how they contribute to cell function. Especially for the last question, it would be highly desirable that inhibitors were available that would be specific for particular TRP channels. Unfortunately, this is not the case. Closest to specific blockers come, in some experimental settings, polyvalent cations of the lanthanide series (Zitt et al. 2002). Even though most channels of the TRP family may be blocked by lanthanides (Jung et al. 2003; Lee et al. 2003a), some channels exhibit a fairly high sensitivity for lanthanides such as gadolinium that exceeds by far that of other channels of the family (Halaszovich et al. 2000) or cation channels in general (Hirasawa et al. 2000; Enyeart et al. 2002). Hence, some specific inhibition can be achieved, at least in special experimental conditions. There are also some reports on successful block of some TRP channels by organic compounds. For example, the non-steroidal anti-inflammatory compound flufenamic acid (FFA) has been described as an inhibitor of the channel TRPM2 (Hill et al. 2004). In the literature, many other effects have been attributed to FFA including block (Tesfai et al. 2001; Lee et al. 2003b; Guinamard et al. 2004) or even stimulation of some TRP channels (Inoue et al. 2001; Pocock et al. 2004). Further targets of fenamates like FFA include chloride channels (Kim et al. 2003), voltage gated Na^+ and K^+ channels (Lee and Wang 1999) and $GABA_A$ receptors (Sinkkonen et al. 2003); in part the effective concentrations were similar as on TRPM2. Thus, FFA should be considered at best a starting point for the development of channel blockers that might be useful not only in the lab but in future also in clinical settings. Similar reservations apply to other organic blockers such as LOE908 (Krautwurst et al. 1993; Krautwurst et al. 1994) or SKF96365 (Merritt et al. 1990).

The molecular mechanisms of Ca^{2+} influx in neutrophil granulocytes and the role of TRPM2

Our own group has been interested now for several years in the role of TRP channels in human neutrophil granulocytes. Fortunately, the results of our and others' research provide a much clearer picture of the situation than in most other cells. In particular, neutrophil granulocytes seem to be the first non-excitable cells in which the molecular basis of a major receptor-mediated Ca^{2+} entry pathway has been identified and in which this pathway's regulation has been principally elucidated, along with the demonstration that the pathway significantly contributes to the function of the cells.

When the expression of genes of the TRP family is studied in neutrophils with RT-PCR experiments, TRPM2 is consistently found with strong signals (Heiner et al. 2003a). The same holds true in HL60 cells although the expression pattern of other TRP channels differs remarkably in this cell culture model of granulocytes in comparison to granulocytes isolated from the blood of volunteers. Admittedly, RT-PCR does not allow to quantify the strength of expression, particularly not in cells in their final state of differentiation such as neutrophils. Nevertheless, these experiments prompted us to seek evidence for the functional expression of TRPM2. The functional hallmark of TRPM2, a member of the melastatin subfamily (Harteneck 2005) within the TRP family, is its activation (or gating) by intracellular ADP ribose (ADPR; Perraud et al. 2001). ADPR probably acts by binding to the Nudix box of TRPM2, an intracellularly located homology motif of pyrophosphatases (see section "Competition with ADPR binding to the Nudix box"). Indeed, when ADPR is infused into neutrophil granulocytes in whole-cell patch-clamp experiments, cation currents carried by Na^+ and Ca^{2+} are consistently induced (Heiner et al. 2003a). These currents can clearly be attributed to TRPM2. This is because TRPM2 in inside-out patches from TRPM2-transfected HEK293 cells shows a characteristic "fingerprint" in form of extremely long channel openings of several seconds and a single channel conductance of 50–70 pS. Such a channel activity can also be resolved in whole-cell recordings from granulocytes, demonstrating that TRPM2 exhibits the same channel properties in these cells as in expression systems. It should be noted that such an identical behaviours of channels in native cells and expression systems is by no means common but rather an exception within the TRP family of cation channels (Heiner et al. 2003b; Plant and Schäfer 2005; Philipp et al. 2005; Groschner and Rosker 2005).

Another mode of TRPM2 activation is oxidative stress for which application of hydrogen peroxide (H_2O_2) is an experimental paradigm (Hara et al. 2002; Wehage et al. 2002). H_2O_2 acts from the intracellular side after permeation through the plasma membrane and uses mechanisms so far undefined in its molecular details but distinct from the mechanism involved in ADPR gating. This conclusion is based on a splice variant (TRPM2-ΔC, see section "Competition with ADPR binding to the Nudix box") that is sensitive to H_2O_2 but insensitive to ADPR (Wehage et al.

2002). Since neutrophil granulocytes exert extreme oxidative stress on themselves during the oxidative burst, this activation mode is probably important during the final and irreversible phase of neutrophil activation.

As third TRPM2 activator, intracellular NAD has been reported (Sano et al. 2001; Hara et al. 2002). Although NAD may bind to the Nudix box as ADPR, it may also be considered that it is intracellularly converted by several pathways (Fig. 1) to ADPR. So far, no TRPM2 variants or artificial mutants are known that display differential sensitivity to ADPR and NAD. Gating of TRPM2 by NAD is somewhat inconsistently observed in expression systems but infusion of NAD into neutrophil granulocytes consistently induces cation currents characteristic for TRPM2 (Heiner et al. 2003a).

To address the question whether gating of TRPM2 by ADPR represents a physiological relevant mechanism for the induction of Ca^{2+} entry and Ca^{2+}-dependent cell activation, we established an HPLC-based method to determine intracellular ADPR levels (Heiner et al., manuscript submitted). From our measurements and from realistic estimations of the intracellular distribution space of ADPR, ADPR concentrations in the range of 2–20 μM are present in unstimulated neutrophils as well as in cells stimulated with *N*-formyl-methionyl-leucyl-phenylalanine (fMLP). Although there was no significant increase of ADPR concentrations in response to fMLP, endogenous ADPR is likely to have an essential role in the cellular response to fMLP. This is because ADPR gates TRPM2 in cooperation with intracellular Ca^{2+} (McHugh et al. 2003). At low intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$), ADPR concentrations in excess of 100 μM are required for a full TRPM2 activation, whereas physiological ADPR concentrations exert hardly any effects. When $[Ca^{2+}]_i$ is raised to 1 μM as may be physiologically achieved by fMLP through $InsP_3$-mediated mobilisation of intracellular calcium stores, considerably lower ADPR concentrations become effective on TRPM2. Indeed, patch-clamp experiments revealed that almost maximal TRPM2 currents are induced in the presence of 5 μM ADPR along with 1 μM Ca^{2+}, which represents conditions easily attainable in fMLP-stimulated granulocytes. Importantly, this activation of TRPM2 channels in granulocytes is not evoked by Ca^{2+} alone but is strictly dependent on the presence of ADPR (Heiner et al., manuscript submitted). Thus, the ADPR–Ca^{2+} system represents a novel signalling principle for Ca^{2+} influx which physiologically takes place in granulocytes and possibly in other cell types as well.

As a preliminary test to which extent ADPR–Ca^{2+}-dependent Ca^{2+} influx contributes to the total fMLP-induced $[Ca^{2+}]_i$ signal, we performed experiments with cibacron blue (Kim et al. 1993; Li et al. 2002a), also named 3GA. 3GA is an inhibitor of NAD glycohydrolases that convert NAD to ADPR (Fig. 1). Preincubation with 3GA for at least 20 min largely (but not completely) depressed fMLP-induced Ca^{2+} entry (unpublished data from this laboratory). For comparison, we performed experiments with the inhibitor BTP2. BTP2 has been demonstrated to abrogate the Ca^{2+} selective current I_{CRAC} in T-lymphocytes (Zitt et al. 2004) that is the prototype of a current induced as consequence of depletion of intracellular calcium stores. The mechanism by which BTP2 exerts this effect is not entirely clear; it is difficult to understand why BTP2 requires a prolonged preincubation time. The compound does probably not block the still elusive I_{CRAC} channels directly. In granulocytes, BTP2 in concentrations of 1 μM (considerably higher than the IC_{50} of 10 nM in lymphocytes) and a preincubation time of 2 h inhibited Ca^{2+} influx incompletely (Fig. 2a). Ca^{2+} influx was studied in a Ca^{2+}-readdition protocol after fMLP stimulation. Increases in $[Ca^{2+}]_i$ attributable to fMLP induced Ca^{2+} influx were depressed by about 60% by BTP2 and to roughly the same extent by 3GA (Fig. 2b). These results let us conclude that the 3GA-sensitive Ca^{2+} influx contributes sizably to receptor-mediated Ca^{2+} entry in neutrophil granulocytes, no less than the BTP2-sensitive influx that may contain the store-dependent component.

ADPR appears not only essential for Ca^{2+} influx induced by fMLP but also for the influx induced by the chemokine interleukin-8 (IL-8). Similar to the fMLP receptors, the IL-8 receptor is a heptahelical membrane protein coupled to G proteins but previous results (Partida-Sanchez et al.

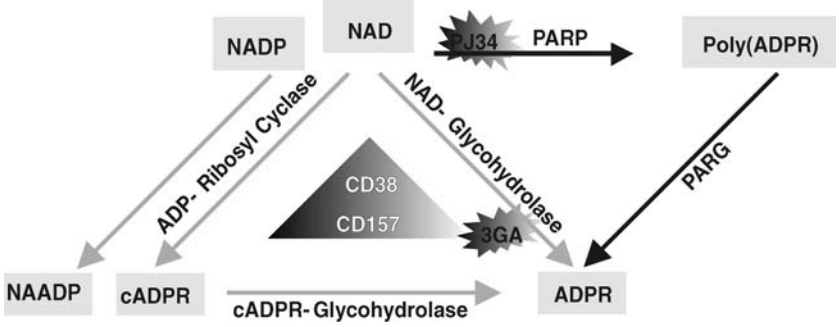

Fig. 1 Schematic representation of metabolic pathways connecting the TRPM2 activators NAD and ADP ribose (ADPR). NAD may be converted directly to ADPR by NAD glycohydrolases. There are two indirect alternatives: either via cADPR, or via Poly(ADPR). CD38 and its close homologue CD157 may act as NAD glycohydrolase, as ADP-ribosyl cyclase, and as cADPR-glycohydrolase. ADP-ribosyl cyclases may also convert NADP to NAADP. Note that NAADP and cADPR, in contrast to ADPR, did not evoke channel gating of TRPM2 (Perraud et al. 2001). *NAADP* nicotinic acid adenine dinucleotide phosphate; *cADPR* cyclic ADPR; *PARP* poly (ADP-ribose) polymerase; *PARG* poly(ADP-ribose) glycohydrolase; *PJ34* a PARP inhibitor; *3GA* cibacron blue, an inhibitor of NAD glycohydrolases. With modifications from (Lee 2001; Schuber and Lund 2004)

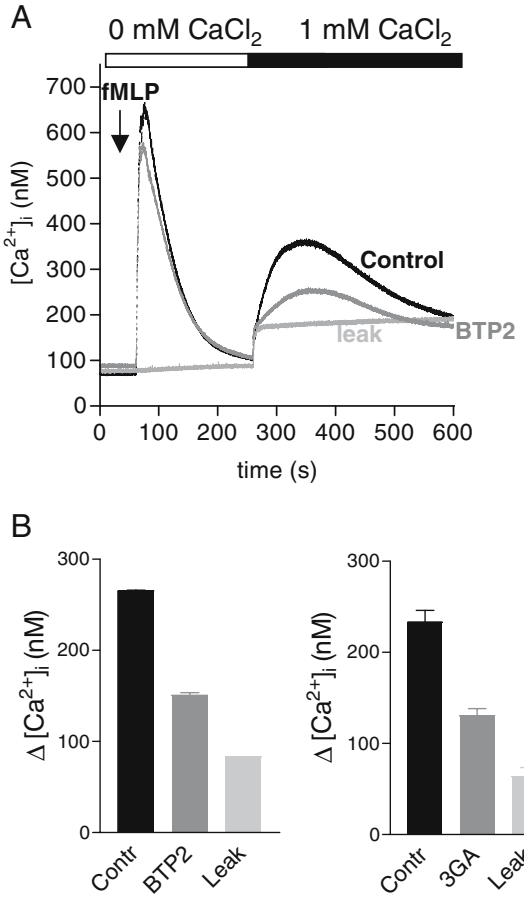

Fig. 2 Effects of the inhibitor BTP2 on the Ca^{2+} influx in neutrophil granulocytes after stimulation with fMLP, in comparison to the effects of 3GA. Cells were preincubated with 3GA (100 μM), an inhibitor of glycohydrolases, for 30 min or with BTP2 (1 μM), an inhibitor of store-operated Ca^{2+} influx, for 120 min. Stimulation with fMLP (1 μM) was performed in the absence of extracellular Ca^{2+}; Ca^{2+} readdition (to 1 mM) was performed 200 s later. **a** Original traces of $[Ca^{2+}]_i$ measurements with fluo-3, deriving from three different experimental conditions, are superimposed: controls not preincubated with an inhibitor (*black*), cells preincubated with BTP2 (*grey*), or cells not exposed to any stimulus or inhibitor, which were used to assess "leak" Ca^{2+} influx (*light grey*). **b** Summary of the experiments shown in **a**, displaying the effects on increases in $[Ca^{2+}]_i$ induced by Ca^{2+} readdition (*left*), compared with the inhibition evoked by 3GA (*right*). After correction for the leak, significant inhibitions were induced by both inhibitors ($n \geq 3$, error *bars* represent ± SEM).

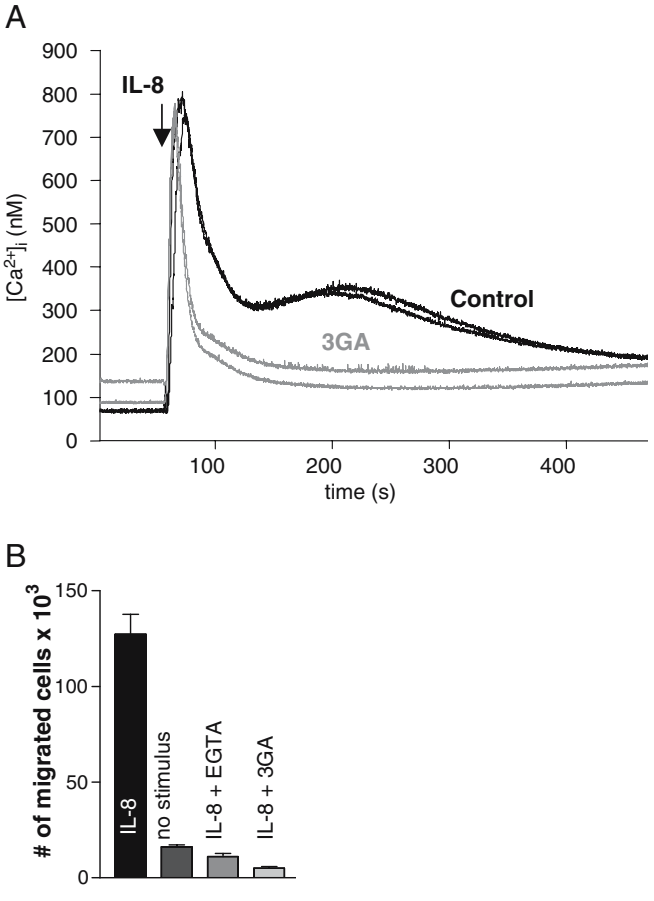

Fig. 3 Effects of 3GA on IL-8-induced Ca^{2+} influx and chemotactic migration in neutrophil granulocytes. **a** Superimposed traces from $[Ca^{2+}]_i$ measurements, without (*black*) or after preincubation with 3GA (*grey*). The extracellular Ca^{2+} concentration was 1 mM. 3GA was removed from the bath prior to the measurements because of its interference with fluo-3 fluorescence. **b** Cells migrated in a transwell assay, with or without stimulation by IL-8, and in stimulated cells in the presence of either 3GA or the Ca^{2+} chelator EGTA. The IL-8 concentration was 10 nM. Each *bar* represent triplicates. The same findings were obtained in triplicates from a further experiment.

2001) have suggested that it may have a differential link to ADPR production (see also section "Inhibition of glycohydrolases and of CD38"). A significant depression of IL-8-induced Ca^{2+} influx was evoked by 3GA (Fig. 3a). It has to be studied in future whether IL-8 raises ADPR concentrations or whether basal ADPR concentrations are sufficient for IL-8-induced Ca^{2+}-mediated TRPM2 gating.

One prominent enzyme in granulocytes that produces ADPR (Fig. 1) is the transmembrane glycohydrolase CD38 (Partida-Sanchez et al. 2004). Paradoxically, CD38 is an ectoenzyme for which the conversion of the substrate (i.e. NAD) to the product ADPR takes place extracellularly (Howard et al. 1993). It remains unclear if CD38 may be translocated intracellularly by stimulation. Alternatively, ADPR formed extracellularly could be transported through the plasma membrane and stimulate TRPM2 as intracellular messenger after its uptake. Indeed, we have obtained preliminary evidence for such a mechanism by experiments measuring the uptake of radioactivity after extracellular application of ^{14}C–NAD. Moreover, fMLP-induced Ca^{2+} influx was enhanced by extracellular application of NAD and of ADPR (Heiner et al. manuscript in preparation). Unfortunately, a more detailed characterisation of the process appears without reach at present due to the lack of specific inhibitors.

ADPR-induced Ca^{2+} influx is essential for chemotaxis

In an important extension of our work on the relevance of ADPR for Ca^{2+} influx, we have demonstrated that ADPR by its induction of Ca^{2+} influx bears functional relevance

for neutrophil granulocytes. As test for function, we performed a "transwell assay" (Wilkinson 1988) that assesses the chemotactic migration in response to IL-8. Migration in granulocytes is a Ca^{2+}-dependent process; since the migration is allowed to proceed over 45 min, releases of Ca^{2+} from stores is not sufficient but Ca^{2+} influx becomes essential. Strikingly, addition of 3GA to the cells completely prevented any IL-8 induced migration. The same effect was obtained when Ca^{2+} influx was abolished by chelation of extracellular Ca^{2+} with EGTA (Fig. 3b). Thus, ADPR as novel mediator and TRPM2 as Ca^{2+} influx pathway have an essential role in the process of neutrophil activation.

Prospective pharmacological targets to modulate Ca^{2+} influx-dependent neutrophil activation

As discussed in the previous chapters, a key role in the activation of neutrophil granulocytes is played by TRPM2 channels and their activation by ADPR, probably produced extracellularly by CD38 and transported through the plasma membrane into the cells. Our level of understanding of these processes opens the possibility that neutrophil activation may be pharmacologically modulated in a specific and effective way and that this may prove clinically useful in the future. For a discussion of the pharmacological perspectives, it should be kept in mind that any potential drugs may be applied either systemically or locally, for example into the synovial fluid in arthritic conditions. Potential molecular targets are not only the TRPM2 channel and its pore but moreover, and most likely preferentially, the production of ADPR, its transport into the cells and the gating process of TRPM2 by ADPR that probably involves binding of ADPR to the Nudix box of TRPM2.

Little hope is for the development of a clinically useful specific channel blockers that would "plug" the channel pore. Besides the usual problems of cross-sensitivity within the TRP family, it should also be considered that TRPM2 is expressed in many different cell types. Even though the roles of TRPM2 have not been clarified in many of them, any other than strictly local application is likely to be associated with undesired systemic side effects. As alternative strategy, drugs may be devolved that are directed against the activation mechanisms upstream of TRPM2. Some of these mechanisms appear sufficiently unique for neutrophils to allow a specific targeting.

Inhibition of glycohydrolases and of CD38

As noted, an important enzyme responsible for the production of the TRPM2 activator ADPR in neutrophil granulocytes is the membrane-associated glycohydrolase CD38. CD38 is common to several types of blood white cells including monocytes and lymphocytes (Mehta et al. 1996). There exists a CD38 knock-out mouse that displays disturbed Ca^{2+} handling of granulocytes, associated with a diminished chemotactic response to the bacterial chemoattractant peptide fMLP (Partida-Sanchez et al. 2001). Surprisingly, no such diminished response was seen when IL-8 was used as a stimulus. There appear to exist some distinct differences between murine and human granulocytes with respect to the differential signalling of fMLP and IL-8. In particular, IL-8 induces a considerably less sustained $[Ca^{2+}]_i$ signal than fMLP in murine cells, whereas both stimuli evoke equally prolonged increases in $[Ca^{2+}]_i$ in human cells (Fig. 2a). Furthermore, in murine neutrophils, the fMLP-induced chemotaxis seems to depend on formation of cyclic ADPR (cADPR) rather than on the non-cyclic ADPR effective on TRPM2 since in these cells chemotactic migration as well as fMLP induced increase in $[Ca^{2+}]_i$ were reduced by 8-Br-cADPR, a membrane permeant cADPR antagonist (Partida-Sanchez et al. 2001). In contrast, in human neutrophils both the Ca^{2+} signal and the chemotactic response towards fMLP were not affected by 8-Br-cADPR (Partida-Sanchez et al. 2004). Therefore, the results on murine neutrophils do not necessarily completely represent the situation in human granulocytes. If CD38 were in fact an exclusive mediator of responses to bacterial peptides but not to endogenous cytokines, the potential usefulness of CD38 would have to be interpreted with more reservation than if CD38 had a uniformly obligatory role. Nevertheless, we consider CD38 inhibition as a promising target. Due to the extracellular localisation of the enzymatic side of CD38, hydrophilic membrane-impermeant inhibitors could be employed that for example might allow a long-lasting local control of granulocyte activation in inflamed joints. So far, cibacron blue is one substance that has been shown to inhibit glycohydrolases in vitro and could be used as a prototype for drug design.

Inhibition of nucleoside uptake

Even more interesting than an inhibitor of ADPR formation would be any potential inhibitor of ADPR uptake into the cell. This is not only from the view of a researcher who would like to use it as pharmacological tool to study the molecular nature and biological relevance of such an uptake. Hence the transport has not been elucidated in its molecular details so far. Only basic features have become evident: it seems to be a high capacity transport non-saturable with ADPR concentrations in the millimolar range. Moreover, it is a regulated process relevantly stimulated by or even completely dependent on fMLP (Heiner et al., manuscript in preparation). However, since ADPR and NAD may be extracellularly interconverted to each other, the substrate specificity is unclear. In particular, our preliminary experiments would be consistent with the possibility that NAD is no less transported than ADPR.

Competition with ADPR binding to the Nudix box

The specific interaction between the channel activator ADPR and the NUDT9 homology region of TRPM2 represents a further potential pharmacological target (Fig. 4). The NUDT9-homology region, located in the C-termi-

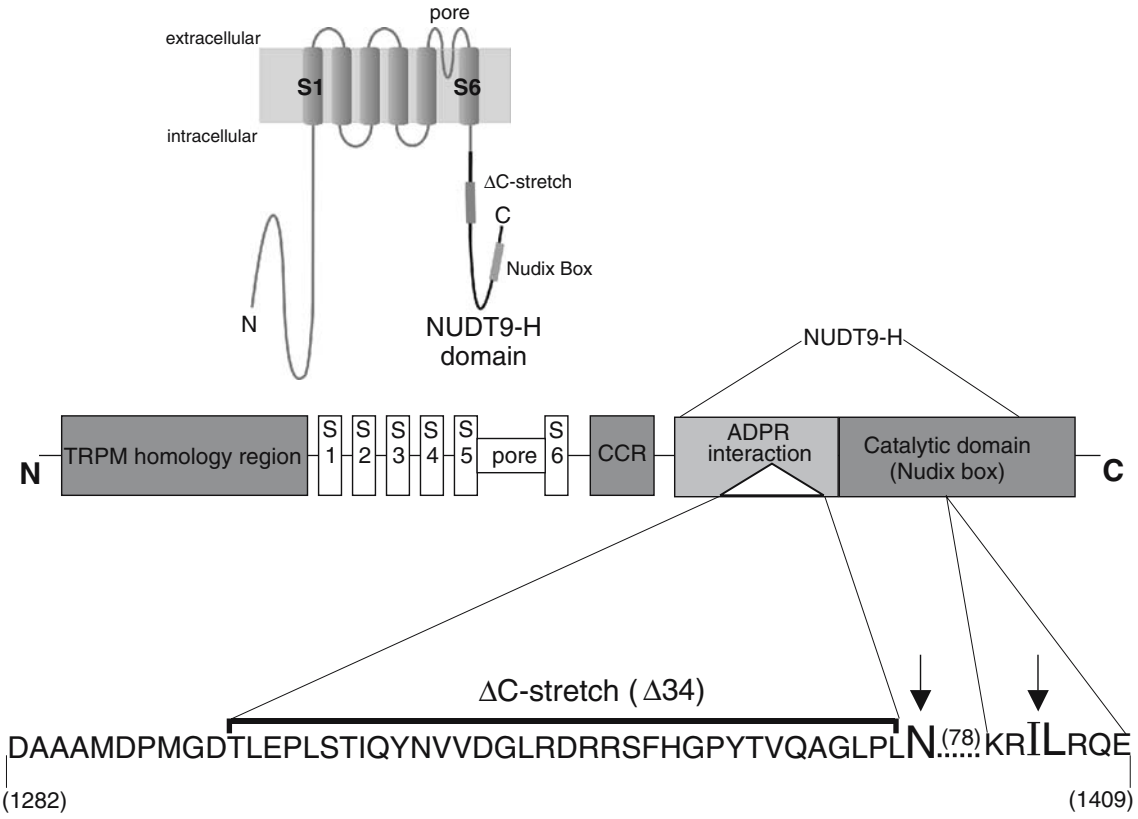

Fig. 4 Schematic representation of the TRPM2 channel structure with emphasis on the C-terminal domain interacting with ADPR. Important parts within the structure of TRPM2 include the TRPM homology region, the transmembrane spans S1–S6 with the pore between S5 and S6, the coiled coil region (CCR), and the C-terminal NUDT9-homology region. The NUDT9-H region (*black* in contrast to the *grey* of the rest of the protein) consists of an *N*-terminal domain that directs ADPR binding and a C-terminal catalytic domain, the Nudix box. The ΔC-stretch within the binding domain is missing in a ADPR-insensitive splice variant. *Arrows* point to the amino acid residue N1326 essential for the putative ADPR binding domain and the two neighbouring amino acid residues IL1405/1406 within the Nudix box; these two residues are probably responsible for the diminished (eliminated?) enzymatic activity of TRPM2 in comparison to pyrophosphatases.

nal cytosolic tail of TRPM2, contains 268 amino acid residues and shows 39% sequence homology to the human NUDT9 ADPR pyrophosphatase (Shen et al. 2003). This protein belongs to the enzyme family of Nudix hydrolases that accept a wide variety of substrates containing a nucleoside diphosphate linked to some other moiety (Perraud et al. 2001). It was by the strong homology of TRPM2 sequences to the Nudix box motif that the activation of the channel by ADPR was discovered (Perraud et al. 2001). ADPR, not to be confused with the cyclic compound cADPR, had previously not been within the catalogue of potential intracellular messengers involved in the gating of ion channels.

The refined crystal structure analysis as well as biochemical studies indicate (Fig. 4) that the human NUDT9 enzyme can be functionally divided into a *N*-terminal part of 105 amino acid residues which supports ADPR binding and a part of 179 C-terminal residues containing the catalytic active site, the so-called Nudix box (Shen et al. 2003). Recently, we have identified a splice variant of TRPM2 (TRPM2-ΔC) in neutrophil granulocytes which is insensitive to ADPR but develops typical cation currents in the presence of H_2O_2 (Wehage et al. 2002). In TRPM2-ΔC, 34 amino acid residues (the ΔC-stretch) are missing within the "ADPR-binding" domain of the NUDT9 sequence motif (Perraud et al. 2003). Further studies of our lab revealed that none of the deleted amino acid residues but a single asparagine residue immediately downstream of the ΔC-stretch is essential for ADPR gating of TRPM2 (Kühn and Lückhoff 2004).

An open question of the field is whether an enzymatic activity of TRPM2 exists, which would make TRPM2 a true "chanzyme", i.e. a channel that represents an enzyme at the same time as is apparently the case for the related TRPM6 and TRPM7 that display kinase activity (Gudermann et al. 2005). Furthermore, it is not clear if such a hypothetical enzymatic activity of TRPM2 may govern channel gating. In the Nudix box of TRPM2, two neighboring amino acid residues (Fig. 4) are changed in comparison to the NUDT9 pyrophosphatase. If the same change is introduced into this enzyme, the catalytic activity is dramatically diminished (Shen et al. 2003). If the change is reverted in TRPM2, loss of channel activity ensues (Perraud et al. 2004; Kühn and Lückhoff 2004). These results strongly suggest that channel activation requires prolonged binding rather than degradation of ADPR. Therefore, substances that interfere with ADPR binding to the NUDT9 domain would be expected to inhibit chan-

nel gating, in response to ADPR and probably to NAD as well. Only activation by oxidative stress may still be possible. Whether the latter mechanism has any physiological or pathophysiological role is not known. So far, activation of TRPM2 by free radicals has been identified as the mechanisms by which alloxan leads to the destruction of pancreatic β cells (Herson et al. 1999; Inamura et al. 2003), explaining why alloxan can be used to induce diabetes mellitus in experimental animals. In granulocytes, gating of TRPM2 by free radicals is likely to take place during the oxidative burst. However, this happens late in the process of granulocyte activation, whereas the action of ADPR takes place earlier and enhances the Ca^{2+}-dependent initiation of the oxidative burst. Therefore, drugs that target the Nudix box are expected to be effective inhibitors of neutrophil activation. Their potential draw back, as in the case of direct channel blockers, is the general inhibition of TRPM2 activation in many other cells than granulocytes. In contrast to hypothetical CD38 inhibitors that could act extracellularly, Nudix box "blockers" had to be membrane-permeant and might therefore be more difficult to design if they were to be used as local, for example intraarticular drugs.

Inhibition of PARPs

It should be noted that another principle of how TRPM2 activation may be prevented has been reported. This is the inhibition of poly(ADP-ribose) polymerases (PARPs; (Fonfria et al. 2004). PARPS are interesting in the context of TRPM2 because of their link to oxidative stress since they lead to the accumulation of ADPR polymers in response to oxidative DNA damage; these polymers are then degraded to ADPR by poly(ADP-ribose) glycohydrolases (PARGs; Fig. 1; Ame et al. 2004). Up to 18 subtypes of PARP homologues are known. One inhibitor, PJ34 with proven activity on PARP1, dramatically diminished Ca^{2+} entry through TRPM2 in HEK293 cells stable transfected with TRPM2 (Fonfria et al. 2004). The role of PARPs for TRPM2 activation is incompletely understood. In granulocytes, PARP1 is little or not at all expressed (Sanghavi et al. 1998). Preliminary experiments in our lab revealed that PJ34 attenuated Ca^{2+} entry in neutrophil granulocytes to an only minimal, hardly significant extent. The effects of PARG inhibitors on TRPM2, which would be expected to be similar as those of PARP inhibitors, have not yet been published.

Perspectives

Within the growing TRP family, TRPM2 is probably the channel for which the mode of activation has been best understood and for which no obvious discrepancies exist between experiments in heterologous expression system and studies of the native channel. Moreover, TRPM2's role in neutrophil granulocytes has become evident and its cell-specific regulation has been elucidated in part. Thus, even though many aspects still need to be clarified, particularly any potential regulation of ADPR formation and uptake, the scientific basis has been founded on which a rational development of drugs can be started, with the aim of a future clinically beneficial modulation of granulocyte function. It is hoped that useful pharmacological tools will become available for basic research much earlier and that these tools will improve the ongoing studies on the molecular mechanisms and biological relevance of TRPM2 activation.

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