Non-Selective Cation Channel Blockers: Potential Use in Nervous System Basic Research and Therapeutics

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Abstract: Non-selective cationic channels (NSCC) are a heterogeneous family of channels, widely expressed in non-excitable and excitable cells, that share several functional characteristics but have diverse molecular origin. NSCC can be formed by transient receptor potential (TRP) channels, calcium activated non-selective channels, hyperpolarization activated cation currents, acid-sensitive cationic channels (ASIC), etc. As a result of its wide expression, as well as to the fact that the activation of such currents produce a persistent membrane depolarization, NSCC have been involved in a variety of neuronal processes such as signal transduction, firing pattern (including plateau potentials and bursting mechanisms) as well as synaptic transmission. Due to the relevance of such channels, alterations in their normal function have been associated with the pathophysiology of several nervous system diseases. Over the last years several blockers of such channels have been discovered. Here we review the pharmacology of NSCC blockers including trivalent cations, verapamil derivates, flufenamic acid, the "typical" TRP blockers 2-APB, ACA and SKF 96365 as well as ASIC blockers. This review focuses on the pharmacological properties of such drugs and their potential use for the understanding of the nervous system as well as for the treatment of neurological diseases.

INTRODUCTION

Neuronal activity relies on the opening and closing of ion channels, which are transmembrane pores that allow the passage of ions through the cellular lipid bilayer [1-3]. Approximately 300 ion channels have been predicted in the human genome and the ones described so far have been classified, physiologically or molecularly, based upon their permeability to different ions, conductance, gating mechanism, voltage dependency and sequence similarity [1-3]. Included in this ion channels diversity, there is a family of cationic channels that, based on the fact that produce an ionic conductance constituted by a mixture of several cations (Na^+, K^+ and Ca^{2+}), have been called non-specific cation channels (NSCC). Members of this family include ion channels with diverse molecular origin such as transient receptor potential channels (TRP), calcium activated non-selective channels (ICAN), acid-sensitive cationic channels (ASIC) and hyperpolarization-activated cation channels (HCN) [4-8].

NSCC are expressed in all animal tissues and participate in a diversity of functions in both excitable and nonexcitable cells [9-13]. NSSC are particularly involved in many neuronal functions such as thermosensation, nociception, osmosensation, chemosensation, phototransduction, firing patterning, pacemaker activity, synaptic transmission, etc. [4, 12,14-18]. Therefore alterations in the activity of such channels have been associated with several nervous system pathologies such as epilepsy, schizophrenia, bipolar disorder and several neurodegenerative disorders such as stroke, Parkinson´s disease, amyotrophic lateral sclerosis and Alzheimer disease [19-26]. Here we briefly review the basic characteristics of different members of the NSCC family as well as the pharmacological tools available for the study of such channels (Fig. **1**).

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Transient Receptor Potential Channels (TRP Channels)

TRP channels constitute a superfamily of NSCC sharing a structure very similar to the TRP channel originally reported in the fruit fly [4, 27, 28]. From the discovery of these proteins in *Drosophila melanogaster* [29, 30], more than 30 mammalian homologous genes have been cloned and their biophysical and pharmacological properties have been studied in the last two decades [4, 27, 28]. TRP channels have been grouped in 6 subfamilies called: Canonical (TRPC), Vallinoid (TRPV), Melastatin (TRPM), Polycystyn (TRPP), Mucolipin (TRPML) and Ankyrin Transmembrane Protein 1 (ANKTM1). All those channels contain six transmembrane segments (S1-S6) with a pore region (between S5 and S6); are weakly voltage-dependent and are permeable to calcium, sodium and potassium, with the exception of TRPM5 and TRPM6 that are permeable to magnesium as well [4, 27, 28]. TRP channels have been found in many organs, mainly in the brain but also in heart, kidney, testis, lung, liver, spleen, ovaries, intestine, prostate, placenta, uterus, and vascular tissues [31].

TRP channels participate in several processes in the CNS [for review see 32], such as neurite outgrowth [33], receptor signaling [34, 35], nociception [36, 37], mecanosensation [38], chemosensation [39], pheromone sensation [40, 41] and synaptic transmission [42]. On the other hand, in the peripheral nervous system TRP channels have been involved in responses to temperature, pressure and to inflammatory agents [38, 41, 43, 44]. In view of the broad range of functions relying on TRP channels, it is not surprising that genetic alterations of such channels might produce channelopaties [for a review see 45].

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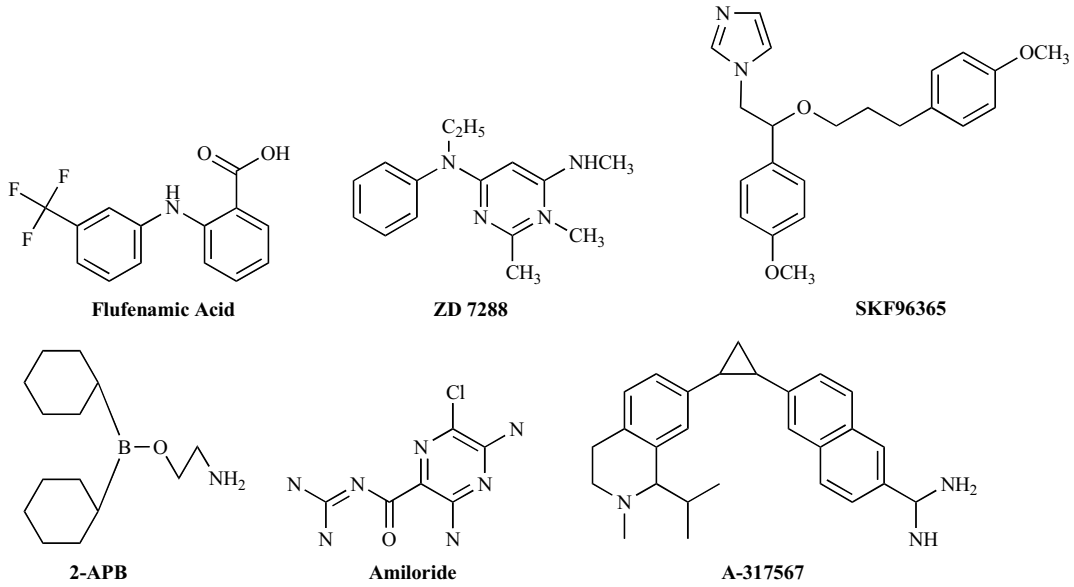

Fig. (1). Chemical structures of the compounds used as non-selective cation channel blockers.

Calcium Activated Non-Selective Currents (ICAN)

The calcium activated non-selective currents (ICAN) are produced by NSCC that activate depending on increases in intracellular calcium concentration [46, 47]. ICANs were first described in cardiac Purkinje fibers and subsequently found in many cells, including neurons [5, 6]. These NSCC produce a cationic conductance of 25-35 pS that is blocked by intracellular adenosine nucleotides such has ATP, ADP and AMP [48, 49]. Despite that the molecular origin of the ICAN have not been completely unrevealed, two members of the TRPM family, TRPM4 and TRPM2, share some of the features described for the ICAN [50, 51].

ICAN has been reported to be present in pyramidal CA1 neurons where it gets activated secondarily to the activation of group I metabotropic glutamate receptors and the release of intracellular Ca^{2+} stores [52]. Recently it has been described that a group of respiratory pacemaker neurons localized in the pre-Bötzinger complex (PBC), rely on the rhythmic activation of an ICAN [53, 54]. In rat supraoptic neurons an ICAN has been implicated in generating depolarization afterpotentials and burst discharges [55]. ICAN has been induced by the activation of muscarinic receptors in pyramidal neurons of prefrontal cortex layer V [56]. In pathological conditions, the ICAN seems to be activated during epileptiform discharges and probably participate in sustaining seizure-like events, since a blocker of this current, flufenamic acid, as well as intracellular buffering of Ca^{2+} with BAPTA dramatically reduced such processes [57]. Since the molecular correlates of the ICAN have not been clearly identified, not genetic-pathological condition has been associated with the ICAN yet.

Acid-Sensitive Cationic Channels (ASIC)

ASICs are cationic channels whose activation and inactivation depend on extracellular changes in the concentration of protons (H^+) [7]. Many members of this family have been cloned and expressed functionally [58-61]. All ASICs belong to the degenerin/epithelial Na^+ channel (DEG/ENaC) superfamily, which are NSSC sensitive to amiloride [57, 62]. The membrane topology of each ASIC subunit consists of two transmembrane domains (TM I and TM II), linked by a large extracellular cystein-rich loop [63-66]. In mammals, six different sequences have now been cloned: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4; these proteins are encoded by four genes. ASIC1b and ASIC2b are splice variants of ASIC1a and ASIC2a. Subunits are able to arrange into homo- or heterotetramers to form functional channels [57, 60, 63, 67, 73]. ASIC1a, ASIC2a and ASIC2b subunits have been shown to be abundant in the brain mainly in cerebral cortex, hippocampus, cerebellum, striatum, habenula, amygdala and olfactory bulb [66-69, 72-76]. ASIC-induced currents are carried mainly for Na^+ and Ca^{2+} [57, 77], which induce membrane depolarization and increase intracellular calcium [66, 78]. The main role of these channels in sensory neurons seems to be related to detection of tissue acidosis as well as the response to inflammatory processes [70, 71]. Beyond these two functions ASICs seem to play a role in other neuronal processes such a nociception, synaptic transmission and neuronal excitability [66, 74, 79]. Due to the involvement of ASICs in these basic neuronal processes, it has been shown that such channels participate in very complex functions such as learning, memory and synaptic plasticity [80]. ASICs seem to be involved in pathologic conditions of the nervous system as well, since several neurodegenerative diseases and pathological conditions in the brain produce acidosis including ischemic brain, inflammation, brain trauma, epileptic seizures, which may lead to the activation of ASICs [81-85].

Hyperpolarization- Activated Cation Currents (HCN)

The hyperpolarization–activated cationic currents (HCN), also called I_h, were discovered more than 30 years ago in heart and nerve preparations [8, 86]. HCN are Na^+ and K^+-

permeable channels that participate in pacemaker currents in cardiac cells and neurons. The channels are opened by hyperpolarization to negative membrane potentials (more negative than −50 mV). In addition, they are also modulated by cAMP binding to a consensus cyclic nucleotide binding domain in the carboxy terminus. Binding of cAMP shifts the voltage dependence of activation to more positive potentials and, in fact, it can also directly open the channels [for a review see 17]. This channels are formed by four known subunits, HCN1–4, each of which has six transmembrane segments. The subunits combine to form homomeric or heteromeric tetramers. The activation of the channel produce an inward current that moves the membrane potential towards action potential threshold [87, 88]. HCN seem to be involved in pacemaker activity, in the control of resting potential and membrane resistance, in dendritic integration as well as in the regulation of synaptic transmission (for a review see 17]. The 4 members of this family are expressed in the mammal brain [87, 88]. A role for HCN channels in epilepsy has been widely proposed [89-98].

PHARMACOLOGICAL TOOLS FOR THE STUDY OF NSCC

Over the last years several synthetic drugs have been reported to block different NSCC, such drugs include the boron-derivated compound 2-APB, the imidazol-derivated compound SKF 96365, the bradycardian agent ZD7288 as well as non-steroidal anti-inflammatory drugs (NSAIDs) such as flufenamic acid (Fig. **1**). Beyond these compounds, NSCC seem to be sensitive to inorganic blockers such as trivalent ions, including gadolinium (Gd^{3+}) and lanthanum (La^{3+}), verapamil and its derivates as well as the polycation ruthenium red. Next we are reviewing the pharmacological properties of several of such blockers and the use of them to understand basic mechanisms of neuronal function and its potential in therapeutics.

Flufenamic Acid, *N*-[3-(trifluoromethyl)-phenyl]anthranilic Acid

Flufenamic acid (FFA; Fig. **1**) is a member of the pharmacological family of fenamates. These analgesic molecules are non-steroidal anti-inflammatory agents capable of producing antipyretic and anti-inflammatory effects in the central nervous system [99]. FFA has been used as a blocker of both TRP channels and the ICAN in several preparations [14, 100-103]. The fenamates, flufenamic acid, mefenamic acid and niflumic acid, block Ca^{2+}-activated non-selective cation channels in a variety of cells [104, 105]. ICAN currents recorded in single channel configuration are blocked by FFA (100μM) [106]. FFA (50-1000μM) inhibits several TRP members as well, including TRPC3 [107], TRPC5 [103 108], TRPM2 [109], TRPM4 [110], TRPM5 [110], whereas it increases activity of TRPC6 [111]. At the cellular level low concentrations of FFA (10 μM) inhibits tonic firing in amygdala neurons [112], this effect might be mediated trough the blockade of a NSCC-induced sustained depolarization, since a slightly higher concentration of FFA (100μM) inhibits a TRP-like inward current recorded in the same neurons [100]. Same concentration of FFA reduces the amplitude of the depolarizing afterpotentials (DAPs) in vasopresinergic neurons of the supraoptic nucleus [113]. Moreover, in dopaminergic neurons, it has been shown that an mGluR1-dependent EPSCs is blocked by FFA (100μM) [102], this EPSC might be produced trough the activation of an FFA-sensitive TRP channel since the increase in intracellular Ca^{2+} produced by the activation of the same glutamatergic receptor was blocked by FFA (100μM) along with another TRP channel blockers such as SK 96365 (30-100μM) and 2-APB (30-100μM) [103]. Similarly, mGluR1-mediated EPSCs recorded from cerebellar purkinje neurons were inhibited by SK96365 (30μM) which support the idea that such synaptic currents are produced through the activation of a TRP channel [114].

On the other hand, FFA (500μM) blocks the ICAN recorded in hippocampal CA1 neurons [46] as well as dopaminergic neurons [115]. Recently it has been shown that FFA (200μM) blocks a Ca^{+2} dependent inward current (presumably carried by ICAN) activated through the calcium carried by nicotinic acetylcholine receptors [115]. FFA (10-200μM) inhibits plateau potentials recorded from nigral GABAergic neurons, which along with the Ca^{2+} dependency of such plateaus suggests that the effect of FFA was produced by blocking an ICAN [116]. Interestingly, the respiratory network localized in the preBötzinger complex (PBC), contains a subpopulation of pacemaker neurons that might rely on the activation of an ICAN, since such pacemaker activity, recorded *in vitro*, has been shown to be blocked by both TTX and Cd^{2+} [117] as well as by FFA (500 μM) and La^{3+} (100 μM) [53]. The involvement of an ICAN in respiratory pacemaker activity has been recently suggested to be occurring *in vivo* as well [118]. Recently, it has been shown that a possible molecular source of the ICAN observed in respiratory neurons might be the TRPM4 and TRPM5, which are expressed within the PBC [119].

FFA affects other ionic mechanisms beyond NSCC, for instance FFA inhibits Ca^{2+}-activated chloride currents [120, 121] and interacts with $GABA_A$ receptors in the brain [122, 123]. In addition, FFA has been shown to modulate gap-junction activity in a pH-dependent manner [124] as well as stretch-activated chloric currents in cultured murine microglia [125]. In rat dorsal root ganglion neurons a Na^+ current was inhibited by FFA (100μM) [126] and a human neuronal K^+ channel expressed in CHO cells was blocked by FFA (100μM) with similar effects of other fenamates in the same preparation [127].

Despite all its non-specific effects, fenamates have been used as neuroprotectants for ischemic (glucose/oxygen deprivation) or excitotoxic conditions. For instance fenamates protect the neurons against ischemic or excitotoxic insults [98, 128]. On the other hand we already mentioned that FFA inhibits seizure-like events in the hippocampus [57, 129].

2-Aminoephoxydiphenyl Borate (2-APB)

This boron derivate permeable-membrane reagent (Fig. **1**) originally use as an antagonist of the inositol 1,4,5-triphosphate receptor (IP3-R) [130-133], is one of the most popular TRP channel blockers. The currents produced in heterologous expression systems by TRPC1 [134], TRPC3 [135] and TRPC5 and TRPC6 [136] are blocked by μM concentrations of 2APB. As mentioned earlier the TRP-medi-

ated glutamate-induced synaptic currents are blocked with 2APB (IC_{50} =69μM) [42]. A cooling-induced current in dopaminergic neurons was inhibited with 2-APB (200μM) as well as with another NSCC blocker ruthenium red [100μM; 137]. 2-APB (50μM) also blocks pheromone transduction in vomeronasal neurons suggesting the participation of TRP channels in such process [44]. It is important to mention that some members of TRP channels family are unaffected by 2-APB. For instance currents produced by TRPC5 are just slightly inhibited by 2APB 100μM [136]. In fact, members of the TRPV subfamily (TRPV1, TRPV2, TRPV3) are activated by such drug (100 μM) [138]. Another unspecific actions of 2-APB are the inhibition of gap junction conductances with an IC_{50} of 5-10μM [139, 140]; inhibition of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps with an IC_{50} of 325-725 μM [141]; inhibition of voltage-dependent K^+ channels at 100 μM [142] and inhibition of Ca^{2+}-activated Cl^- current at the same concentration [143]. 2-APB may have some therapeutic potential since it has been shown that inhibits apoptotic cell death induced by the internal Ca^{2+} stores depletion [144], increases the induction of LTP, a cellular correlate of learning and memory [145] and protects neurons against acute ischemia [128]. Other TRP blockers, that share some pharmacological similarities with 2-APB, have been used recently. For instance *N*-(p-amylcinnamoyl) anthranilic acid (ACA), which was initially used as a phospholipase A_2 inhibitor [146], can block native and recombinant TRPM2 mediated currents at concentrations ranging form 1 to 20 μM [147]. 20 μM ACA also blocks recombinant TRPM8 and TRPC6 currents in HEK293 cells [147]. However further investigation is needed to determine the effects of ACA on different NSCC, as well as its non-specific effects on other ion channels.

SKF 96365

This imidazole derivate, (1-(beta-[3-(4-methoxyphenyl) propoxy]-4- methoxyphenethyl)-1H-imidazole) (Fig. **1**), was initially use in platelets and neutrophils to block receptor-mediated calcium entry *via* voltage-gated calcium channels with an IC_{50} value of approximately 10 μM [148]. Moreover, in several preparations SKF-96365 has been used as TRP blocker. SKF-96365 (25 μM) reduces an ATP- and carbachol-activated TRP-like current along with 2-APB 100μM and La^{3+} 1mM [149]. As mentioned earlier, SKF 96365 (100 μM), blocks the mGlurR-induced TRP-mediated EPSC in dopaminergic neurons [102]. Same effect was described at a lesser concentration (30 μM) in cerebellar Purkinje neurons [114]. In cultured hippocampal pyramidal neurons, TRP-mediated Ca^{2+} influx, in this case activated by NMDA, was inhibited by SKF 96365 (3μM). Interestingly in the same neurons LTP was attenuate at identical concentration, suggesting that TRP may play a key role in synaptic plasticity [150]. A TRP-like conductance, induced by dopamine in dorsal raphe neurons, was blocked by SKF96365 (100μM) and 2APB (100μM), involving TRP-like channels in such process [151]. Similarly a conductance induced by BDNF in CA1 hippocampal neurons is blocked by SKF96365 (30μM), suggesting again a TRP-like current, in this case such correlation was confirmed by the fact that small RNA-inference probes against the TRPC3, reproduced the effect observed for SKF96365 [34]. Other studies have shown that SKF96365 has more effects beyond blocking TRP, for instance it has inhibitory effects on K^+ channels in endothelial cells [152], inhibits the sarcoplasmic reticulum Ca^{2+} ATPase in thymic lymphocytes [153], and facilitates nicotinic receptor desensitization [154]. The therapeutic potential of SKF96365 has been poorly explored so far.

It is important to mention that all TRP blockers reviewed before should be considered as "broad-spectrum TRP channel blockers". Current research is undergoing in order to design and test more specific TRP channel blockers. For instance new TRPV1-specific blockers are currently available such as BCTC [155], AMG9810 [156], A-425619 [157], as well as SB-366791 [158, 159] and capsazepine [160, 161]. SB366791 N-(3-Methoxyphenyl)-4-chlorocinnamide is a potent and selective TRPV channel antagonist that inhibits, in a concentration dependent manner (10-300 nM), TRPV1-induced Ca^{2+} response to capsaicin in 132-1N1 cells [158] as well as in trigeminal ganglion cells [159]. Human TRPV1 channels expressed in HEK cells are also inhibited by SB366791 (1μM), with no effect on HCN currents or voltage-gated Ca^{2+} currents [158]. Capsazepine (CPZ), a synthetic analogue of capsaicin, inhibits vanilloid-evoked currents as well [160]. Despite that, in several neuronal preparations, CPZ has been shown to be a potent TRPV1 channel blocker [161, 162], recently it has been shown that capsazepine may modulate other members of the TRP family such as TRPM8 [163, 164], voltage-gated Ca^{2+} channels [165] as well as nicotinic acetylcholine receptors [166]. These observations clearly show that, in order to determine the potential use of these new TRP channels blockers, is still required to perform corroborative studies to determine their pharmacological properties as well as to rule out possible unspecific effects of such drugs.

Verapamil and Verapamil-Derivates

Verapamil (2-(3,4-dimethoxyphenyl)-5-[2-(3,4-dimethoxyphenyl)ethyl-methyl-mino]-2-(1-methylethyl) pentanenitrile) is a phenylalkylamine commonly used as a blocker of L-type voltage-gated Ca^{2+} channel (VGCC) [167]. Verapamil (150μM) can also block TRP3 currents expressed in HEK-293 cells [168] without affecting TRPC5 currents expressed in the same cells [169]. Over the years several verapamil derivates have been developed, including zatebradine, ivabradine and ZD 7288, and some of them have been shown to be very specific HCN blockers (see next)

Zatebradine and Ivabradine

Ivabradine (*S*)-3-(3-(((3,4-dimethoxybicyclo(4.2.0)octa-1,3,5-trien-7-yl)methyl)methylamino)propyl)-1,3,4,5-tetrahydro-7,8-dimethoxy-2*H*-3-benzazepin-2-one and Zatebradine 3-[3-[2-(3,4-dimethoxyphenyl)ethyl-methylamino]propyl]-7,8-dimethoxy-2,5-dihydro-1H-3-benzazepin-4-one; are verapamil-derivates that block HCN-mediated currents in several cardiac preparations [170]. Zatebradine (2 μM) decrease the pacemarker cardiac current, which is mediated by HCN channels, in short Purkinje fibres from sheep hearts [171]. At the neuronal level it has been reported that zatebradine (1 μM) blocks the I_h in mouse dorsal root ganglion neurons [172], as well as in amphibian rod photoreceptors [173]. However zatebradine (10μM) does block other ion currents

such as the Kv1.5-mediated current [174]. Ivabradine, as zatebradine, blocks pacemaker currents in cardiac tissue [170] as well as spontaneous firing in the isolated SA-node fibers [175]. I_h currents mediated either by HCN1 or by HCN4, expressed in HEK cells, are blocked by ivabradine with an IC_{50} of 0.94μM and 2.0 μM respectively [176].

ZD 7288

ZD 7288 (4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride) is another verapamil-derivated drug (Fig. **1**) that has been extensively used as a HCN blocker [177, 178]. Low micromolar concentrations of this agent specifically block both native I_h and cloned HCN channels in a voltage-independent manner [179-183]. ZD 7288 blocks the I_h in dendritic spines of layer 5 pyramidal neurons in the prefrontal cortex [184]. In neurons of the basolateral complex of thalamus, ZD 7288 (50μM) blocks the I_h and suppresses neuronal excitability and the rhythmic burst firing [185, 186]. In primary cultures of dorsal root ganglion neurons ZD 7288 (100μM) also inhibits the I_h [179] which seems to be the case of pyramidal CA1 neurons as well [186]. Regarding its therapeutic potential, ZD7288 (10-100μM) decreases epileptiform activity induced in neocortical brain slices by application of 4AP and GABA-receptor antagonists [19] or in hippocampal slices induced by 0 Mg^{2+} solution [22]. ZD7288 also disrupts thalamic spindle-waves *in vitro* [187]. On the other hand ZD7288 inhibits neuropatic pain and allodynia in rats [188, 189]

Lanthanides

Two members of lanthanides family, Gadolinium(Gd^{3+}) and lanthanum(La^{3+}), has been used for blocking several NSCC, mainly TRP channels [42, 101, 103,190]. Lanthanides are electropositive metals with a main oxidation number of 3+ (trivalent cations). In trivalent form gadolinium (Gd^{3+}) and lanthanum (La^{3+}) have similarities with Ca^{2+} ions respect to size (Radium **Gd^{3+}** 1.05-1.11 Å, **La^{3+}** 1.061 Å **Ca^{2+}** 1-1.06 Å.), bonding, coordination and donor atom preferences [191]. $GdCl_3$ interferes with normal binding of Ca^{2+} to its membrane binding sites and therefore affects Ca^{2+}-mediated membrane function [192]. Studies in lipid bilayers have shown that lanthanides induce structural changes in erythrocytic membranes due to its great affinity for phospholipids [193, 194]. In cultured cortical neurons, the glutamate-induce delayed calcium increase is blocked by La^{3+} (IC_{50} 7.2±3 μM) [42]. Similarly the previously described mGluR-induced TRP-mediated EPSC is blocked by Gd^{3+} (100μM) [103]. Moreover spontaneous firing activity and basal cytosolic Ca^{2+} concentration in acutely isolated midbrain dopamine neurons was inhibited by La^{3+} 10μM, 2-APB (20μM) and SKF 96365 (10μM), suggesting the participation of NSCC in both processes [195]. In cultured rat dorsal root ganglion neurons TRPV2-like heat-activated currents were inhibited by Gd^{3+} and La^{3+} (100μM) [196]. Beyond its effects as NSCC blockers, lanthanides affects other ion channels, for instance $GdCl_3$ blocks several types of voltage-dependence Ca^{2+} channels, such as the L, N and T type channels [197] as well as K^+ channels [198], and even Na^+ channels [199]. So far it has been shown that both Gd^{3+} and La^{3+} may have a potential protective effect against ischemia along with other NSCC blockers [128].

Ruthenium Red

The inorganic dye ruthenium red (RuR) is a polycationic cell biology reagent of high molecular weight. The more accepted formula is $[(NH_3)_5Ru\text{-}O\text{-}Ru(NH_3)_4\text{-}O\text{-}Ru(NH_3)_5]Cl_6 \cdot 4H_2O$). Among its different molecular targets, RuR has been used as blocker of TRP channels. For instance RuR (10μM) inhibits TRPV1 currents in several cells [200-202]. In rat retinal ganglion cells, the light-activated membrane currents are blocked by RuR (20μM) as well as with SKF 96365 and lanthanides [101]. A cholecystokinin-induced TRP like inward current in amygdala neurons was blocked by external application of ruthenium red (20μM) as well as with 2-APB (100μM), Gd^{+3} (100 μM) and FFA (100μM) [100]. In hippocampal CA1neurons a TRP-like current, which is activated in ischemic conditions, is blocked by RuR (5μM), as well as with Gd^{+3}, La^{+3} and 2-APB [128]. In the spinal substantia gelatinosa cells, mEPSCs enhanced by anadamide and capsaicin were blocked by RuR 10μM, suggesting the participation of TRP channels in such synaptic transmission [203]. Despite RuR is used to block TRPV channel, other ion channels are sensitive to this compound. A member of the family of two-pore-domain potassium channels, TASK-3 is selectively inhibited by RuR (0.3 -10μM) [204-206]. The potential therapeutic effects that RuR are shadowed by the fact that this compound produces hyperexcitation and neurotoxicity in the nervous system [for a review see 206]. When RuR is administered intracranially, intracisternally or intracerebroventricularlly produces generalized convulsions and neuronal death [207].

ASICs Blockers

Since ASICs have been involved in several pathological conditions, the study of molecules with the ability to block such channels is growing. ASIC blockers described so far include amiloride (Fig. **1**), a diuretic agent known to block Na+/H+, Na+/Ca2+ exchangers and ENaC [208,209]. Amiloride reversibly inhibits the ASIC currents with an IC_{50} of 10–50 mM [58, 59, 68, 83]. Similarly to its effect on the ASIC currents, amiloride inhibits acid-induced increase of $[Ca^{2+}]_i$ and membrane depolarization [83, 210-212]. Other ASIC blocker is A-317567, a small molecule unrelated to amiloride (Fig. **1**). This drug inhibits the ASIC1a-like, ASIC2a-like, and ASIC3-like currents in rat DRG neurons with IC_{50} of 2–30 μM [213]. Interestingly in the last years two toxins have shown a more specific and potent effects as ASICs blockers, as compared with the two organic compounds just described; such toxins are Psalmotoxin 1 and APETx2 [214]. Psalmotoxin 1 (PcTX1) is a peptide toxin that specifically inhibits the ASIC1a current [215]. It was isolated from the venom of South American tarantula *Psalmopoeus cambridge*. In heterologous expression systems, PcTX1 potently and specifically inhibits the acid-activated current mediated by homomeric ASIC1a subunits in a nanomolar concentration range (IC_{50} < 1 nM); 214]. On the other hand APETx2 is a toxin isolated from the sea anemone *Anthopleura elegantissima*. It is a potent and selective inhibitor for homomeric ASIC3 and ASIC3 containing channels [216]. It reduces acid-evoked currents mediated by homomeric ASIC3 channels in heterologous expression systems and in primary cultures of sensory neurons [216].

Regarding the therapeutic potential of ASIC blockers we can found that amiloride has been shown to suppress acid-induced pain [209, 212], whereas in CNS neurons, it reduces acid-mediated and ischemic neuronal injury [83, 217]. A-317567 reduces thermal hyperalgesia in a rat model [213]. Intracerebroventricular administration of PcTX1 has been demonstrated to be effective in reducing ischemic brain injury in rat and mouse models of ischemia [83].

CONCLUDING REMARKS

In conclusion, considering the information reviewed, is clear that NSCC play a key role in several neuronal functions and represent potential therapeutic targets for diseases of the CNS. Increasing evidence supports the involvement of NSCC activation in physiological processes such as signal transduction, firing pattering and synaptic transmission. It is also clear that NSCC may be involved in neurological diseases mainly in brain ischemia and epileptic seizures. It will be important to determine the specific involvement of particular types of NSCC in several models of pathologies of the SNC. It will be important as well to develop more specific and potent blockers for individual NSCC subunits in order to advance our understanding of the role of these channels in physiological and pathological processes, and for establishing novel therapeutic strategies for neurological diseases. Although none of the pharmacological agents reviewed here is uniquely specific for a member of the NSCC family, these drugs have facilitated the understanding of several neuronal processes and have shown promising therapeutic effects in animal models.

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