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COMMENTARY

SK channels are on the move

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Small-conductance Ca^{2+}-activated K^+ channels (SK channels) underlie the medium duration after hyperpolarization that follows single or trains of action potentials in many types of neurons. Three subtypes of SK subunits, SK1 ($K_{Ca}2.1$), SK2 ($K_{Ca}2.2$) and SK3 ($K_{Ca}2.3$), have been cloned and are expressed differentially within the central nervous system (CNS). A paper in this issue of *BJP* reports the discovery of the first example of a positive modulator displaying not only selectivity for SK channels over other channels, but also a subtype selectivity among SK and analogous channels (SK3 > SK2 ≫ SK1 = IK). Together with other recent progress in the field, this finding enriches the repertoire of tools available to test the hypothesis that SK channels may be targets for future CNS drugs.
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Abbreviation: CYPPA, cyclohexyl-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine

Voltage-dependent Na^+ and Ca^{2+} channels are already exploited as targets for central nervous system (CNS) drugs. However, this is not yet the case for the very diverse class of K^+ channels, where it is more challenging because of their often ubiquitous distribution (Gutman *et al*., 2005; Wei *et al*., 2005). Nevertheless, one step in that direction may have been made in the field of small-conductance Ca^{2+}-activated K^+ channels (SK channels) with a study published in this issue of *British Journal of Pharmacology* (Hougaard *et al*., 2007).

Single or trains of action potentials are followed by an afterhyperpolarization (AHP), which comprises kinetically distinct components (Sah and Faber, 2002). In many types of neurons, SK channels underlie the medium duration AHP that peaks around 40 ms after the action potential(s) and lasts for 200–500 ms. Blockade of this component will increase the firing rate (Stocker, 2004) and/or change the firing pattern (Waroux *et al*., 2005) of the neuron, whereas a positive modulator will decrease its excitability (see below). Three subtypes of SK subunits, SK1 ($K_{Ca}2.1$), SK2 ($K_{Ca}2.2$) and SK3 ($K_{Ca}2.3$) have been cloned and are expressed differentially within the CNS (Köhler *et al*., 1996; Stocker and Pedarzani, 2000). These channels, which consist of homo- or heterotetramers, are exclusively gated by intracellular Ca^{2+}. The effector concentration for half-maximum response (EC_{50}) of Ca^{2+} is about 400 nM, with a Hill coefficient ~4 (Köhler *et al*., 1996). Subsequent experiments have revealed that Ca^{2+} acts by binding to the N-lobe of calmodulin which is constitutively bound to the C terminus of each subunit (Schumacher *et al*., 2001). Finally, recent biochemical experiments have revealed, at least in the case of SK2 subunits, that the channels can reside within a complex multiprotein assembly comprising at least a kinase (CK2) and a phosphatase (PP2) (Bildl *et al*., 2004; Allen *et al*., 2007). An increased phosphorylation of calmodulin residues shifts the response of SK2 subunits to Ca^{2+} to the right (Allen *et al*., 2007).

What about pharmacology? Currently, SK channel modulators include pore blockers, positive and negative modulators. The two last categories work by increasing and decreasing, respectively, the sensitivity of the channels to Ca^{2+}.

Classical blockers include the bee venom octodecapeptide apamin, as well as several related peptides (reviewed by Liégeois *et al*., 2003). Different classes of nonpeptidic blockers have also been synthesized and characterized by the Jenkinson group (Campos Rosa *et al*., 2000) and, more recently, by our group (Graulich *et al*., 2006).

The first example of a negative modulator, NS8593, was reported last year (Strøbæk *et al*., 2006). It shifts the Ca^{2+} concentration–response curve 4- to 5-fold to the right at 3 μM and does not interact at all with ^{125}I-apamin-binding sites.

Recent progress has also been made in the field of positive modulators in terms of potency: after the discovery of the first compound, which works in the mM range, 1-ethyl-2-benzimidazolinone (1-EBIO) (Pedarzani *et al*., 2001), a dichloro derivative, DC-EBIO, as well as a new compound, NS309, were found to modulate the channels in the 10 and 0.1 μM range, respectively (Strøbæk *et al*., 2004).

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When working on ion channel modulators, two main pharmacodynamic issues arise at once: selectivity within the ion channel family (SK1, SK2 and SK3 in this case) and selectivity versus other channels and also receptors.

The paper by Hougaard *et al*. (2007) is a significant progress in the first direction. Indeed, neither EBIO derivatives nor NS903 have any selectivity within SK subtypes. Further, they also act on IK channels, which are Ca^{2+}-dependent K^+ channels that are mainly expressed in nonneural tissue, such as lymphocytes and erythrocytes. In this study, the Neurosearch team reports the characterization of cyclohexyl-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine (CYPPA) as a subtype-selective positive modulator. CYPPA belongs to a different chemical class from the previous compounds. Using various patch-clamp configurations and a fluorescence-based assay on cells stably transfected with human SK subunits, they demonstrate that this compound enhances the sensitivity of SK3 and SK2 homomers to Ca^{2+} without affecting SK1 or IK channels. The compound acts on the channels from the high nanomolar range and its EC_{50} (see their methods for its particular meaning in this case) is 6 and 14 μM for SK3 and SK2, respectively. Thus, as compared with NS309, a significant selectivity is gained with CYPPA, at the expense of potency. Interestingly, the authors show that CYPPA does not interfere with the action of NS309 on SK1 homomers, suggesting that it is not able to bind to the site where NS309 has its action on this subunit. Importantly, the authors tested the selectivity of the compound versus other targets and report that, although several channel subtypes are insensitive to CYPPA, it does block BK and $Na_v1.2$ channels in the low μM range. This may raise some concern about its general use as a pharmacological tool in tissues or whole organisms.

Nevertheless, this elegant study is a clear proof-of-principle demonstration that selectivity can be achieved in the field of positive modulators among the SK subunits. Clearly, additional work is needed to critically assess the potential of this compound as a pharmacological tool. Firstly, it will be important to examine whether or not CYPPA affects SK heteromers, such as SK1/SK2 channels, which have been demonstrated to exist, at least *in vitro* (Benton *et al*., 2003). Work with the compound in brain slices will also be needed to address the selectivity issue versus other targets in a much more complex environment. Finally, further structure–activity work may provide even more specific molecules in the future.

Progress is also being made on the front of the so-called pore blockers. Earlier this year, using various SK chimerae, the Stocker group showed that apamin, but not the nonpeptidic blocker tubocurarine, blocks SK channels by also binding to a region outside the pore vestibule, namely the extracellular loop between S3 and S4 (Nolting *et al*., 2007). Indeed, the blocking potency of apamin for native SK channels is SK2 > SK3 ≫ SK1 and the authors managed to render a hSK1 subunit very sensitive to the peptide by mutating one amino acid in this loop and also three amino acids in the pore region. Very interestingly, the sensitivity to tubocurarine was unaffected. Taken together, the data show that apamin achieves subtype selectivity in part by acting on a region outside the pore. It is not difficult to imagine how this is possible. It is usually assumed that block is imparted by two charged arginine residues, which are in positions 13 and 14 of the peptide. This certainly provides room for the 12 amino-acid chain located upstream to bind elsewhere on the subunits. Another important consequence of this finding is that the so-called pore blockers may be much more heterogeneous in the way they block than was previously thought, both quantitatively and qualitatively.

In summary, much progress is being currently made in the field of SK channels, in terms of pharmacology, medicinal chemistry and channel biophysics. One can only hope that this will eventually lead to the development of selective, systemically active SK channel modulators.

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