However, in a recent study we novel gene that encoded a small K_oocyte expression system to clone a MinK (KCNE1) is a protein, which represent a novel class of proteins, a unifying classification for the pharmaceutical industry when synthesizing new antipsychotic drugs.

As yet, there is very little clinical evidence to support this assumption. However, in a recent study we demonstrated, using PET, an increased dopamine synthesis rate in the medial prefrontal cortex of drug-naive schizophrenic patients, which we interpreted as indicating an increased activity of amino acid decarboxylase because of the lack of a precursor. Indirectly, these results could be interpreted to support the notion of reduced activity of dopamine-containing neurons within the prefrontal cortex in schizophrenia, a hypothesis that was originally put forward by Weinberger et al. If this hypothesis turns out to be verified by further clinical evidence, the study by Herrell et al. might have deep implications for the pharmaceutical industry.

Selected references

Maximal function of minimal K+ channel subunits

Michael C. Sanguinetti

A recent report by Schoeder et al. adds to the intrigue and complexity regarding the function of KCNE proteins, which represent a novel class of β-subunits that modulate the gating of K+ channels. The β-subunits might be very small proteins (103-153 amino acids), but they have a dramatic effect on channel gating. The path of discovery of these new proteins, their functional roles and relationship to human disease is interesting as the associated nomenclature is confusing.

MinK (KCNNE) is a β-subunit

In 1988, Takumi et al. used the Newp oocyte expression system to clone a novel gene that encoded a small K+ channel protein. This protein was named Ik and because it induced a very slowly activating outward K+ current when expressed in oocytes, Ik is more commonly called minK because it is the smallest K+ channel (130 amino acids with a single transmembrane domain) known to be associated with K+-channel function. It was not until 1996 that minK was shown to co-assemble with another subunit (KvLQT1) to form the K<> delayed-rectifier K+ channel. The KvLQT1 gene was discovered by positional cloning techniques after linkage analysis showed that its chromosomal location was linked to long-QT syndrome, an inherited arrhythmia. KvLQT1 is a typical K+ channel subunit that consists of six transmembrane domains and a pore-forming region. Thus, minK is actually a β-subunit that, together with four KvLQT1 α-subunits, forms a single delayed-rectifier K+ channel (K<>).

In an attempt to simplify the confusing nomenclature of channel genes and proteins, a unifying classification has been proposed. MinK is the first member of a new family of proteins called KCNE. Hence, minK protein is called KCNNE1 and its gene is KCNNE1. KvLQT1 is now called KCNQ1, and three other members in this gene family have been described (KCNQ2, KCNQ3). Mutations in any of these genes cause human disease. For example, long-QT syndrome is caused by mutations in KCNQ1 (or KCNFE1). The gene products of KCNQ2 and KCNQ3 co-assemble to form neural M-current channels (K<>), and mutation in either gene causes benign familial neonatal convulsions. Finally, mutations in KCNQ4, which also encodes an M-current-like channel, cause neonatal deafness.

KCNF1 and related proteins modulate KCNQ channel gating

In 1999, new members of the KCNE subunit family were described by Abbott et al. who searched a database for expressed sequence tags for proteins with partial homology to KCNNE1. Three new cDNAs related to KCNNE1 were eventually isolated using reverse transcription-polymerase chain reaction (RT-PCR), and the encoded proteins were named minK-related peptides (MiRP). The genes for the three MiRPs were named KCNFE2-4. KCNFE2-4 co-assemble with an α-subunit human ether-a-go-go-related gene (HERG), to form the rapid delayed-rectifier K+ channel (K<>). HERG is thought to be a tissue-specific gene, which is predominantly expressed in the heart, and associates with HERG and modulate its function.

COMMENT

References

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might not be of physiological impor-
tance because HERG subunits prefer-
entially form complexes with KCNQ2 subunits when they are co-cultivated with KCNE2 and KCNE1 synthesized in vitro14. Because it was known that mutations in either the KCNQ1 or HERG α-subunit genes caused long-
Q-T syndrome19,20, it was perhaps not too surprising to find that mutations in either of the associated β-subunit genes KCNQ1 or KCNE2 also caused this inherited arrhythmia.

The latest chapter in the KCNE sub-
unit story is particularly intriguing. KCNQ1 homomultimeric channels conduct a relatively slow delayed-rectifier K\(^+\) current that has strong voltage-depen-
dent gating. KCNQ1 channels open only in response to positive depolariza-
tions of the membrane to potentials of about \(-40\) mV. By contrast, KCNQ1– KCNE3 heteromultimeric channels are constitutively open, and thus a change in voltage is not required for the chan-
nels to open. It had earlier been shown that KCNE1 subunits greatly slowed the rate of KCNQ1-channel activation. Thus, by mechanisms that still need to be elucidated, the association of KCNE1 or KCNE3 subunits with KCNQ1 sub-
units has dramatically different effects on channel gating. The interaction domains on KCNQ1 and KCNE3 subunits that mediate the change in gating behavior still need to be determined and there is still no general agreement as to how KCNQ1 and KCNE3 subunits inter-
act15,16. In addition to altered gating, several other experimental findings re-
ported by Schroeder et al.17 indicate that KCNQ3 and KCNQ4 proteins interact in a functional manner. (1) The phar-
macology of KCNQ1 channel gating was markedly altered by co-expression with KCNE1, channel were more sen-
sitive to block by chromanol 293B and clotrimazole. (2) The surface expres-
sion of epitope-tagged KCNE3 was enhanced by co-transfection with KCNQ1 in Chinese hamster ovary (CHO) cells. (3) Transcripts for KCNE3 and KCNQ1, detected by in situ hybridization, were localized in crypt cells of the small intestine and, by northern-blot analysis, in ileal epithelial cell line. Ideally, one would prefer to have direct evidence for protein association in native tissue such as colonic crypt cells. However, co-immunoprecipitation of KCNE1 and KCNQ1 subunits in native cells must await the availability of spe-
cific antibodies to the two proteins. Curiously, when co-expressed with either HERG or KCNQ4 (but not KCNQ2 or KCNQ3), KCNQ3 abol-
ished functional expression18. The physiological relevance of these inter-
actions is uncertain, especially in tissues where KCNQ3 is poorly expressed such as the heart and cochlea, whereas HERG and KCNE3 subunits, respec-
tively, are strongly expressed and have known functions.

Physiological role for KCNQ1–KCNE3 channels

Schroeder et al.17 propose that KCNQ1– KCNE3 channel conduct the baso-
lateral K\(^+\) current that hyperpolarizes secretory epithelial cells and thereby increases the secretion of Cl\(^-\) from the apical membrane via cystic fibrosis transmembrane conductance regulator (CFTR) channels. K\(^+\)–dependent, elec-
trogeneic Cl\(^-\) secretion, first described in tachael epithelium by Smith and Frizzel in 1984 (Ref. 17), also occurs in the intestine and colon and requires a K\(^+\) conductance at negative membrane potentials. The voltage-independent open probability of KCNQ1–KCNE3 channels meets this requirement. Heterologously expressed KCNQ1– KCNE3 channels are also stimulated by cAMP and inhibited by both chlo-
ramphenicol 293B and clotrimazole at con-
centrations similar to those that affect the K\(^+\) current of native cells. Together with the finding that transcripts for both subunits co-localize in colonic crypt cells, it seems reasonable to equate KCNQ1–KCNE3 channels with the native K\(^+\) current in secretory epithelial cells of the intestine. However, because the pharmacology of cAMP-activated basolateral K\(^+\) conductance differs between tissues19, it is unlikely that KCNQ1–KCNE3 channels represent the common molecular correlates of this current.

These latest findings, which describe the effect of a KCN1-like protein on K\(^+\) channel gating, emphasize that the functional roles for these minK pro-
teins are anything but minimal. Future experiments should focus on the bio-
physical characterization and structural basis of how KCNQ1 and KCNE3 can have such strikingly different effects on the gating of KCNQ1 channels. In addition, it will be interesting to deter-
mine the functional role of KCNQ1 in tissues where it is most prominently expressed, such as the kidney and small intestine. Given the track record of KCNE and related proteins, it is likely that mutations in KCNE3 will soon be associated with human disease. Finally, the discovery of KCNQ1–KCNE3 channel function should facilitate high-
capacity screening for compounds to treat disorders caused by defective or excessive epithelial Cl\(^-\) secretion.

Selected references

Central 5-HT$_{1A}$ receptors and vagal tone to the airways

In a recent TiPS article, Cazzola and Matera indicated that activation of peripheral 5-HT$_{1A}$ receptors could reduce parasympathetic tone to the airways, therefore implying that 5-HT$_{1A}$ receptor agonists might be useful in the treatment of obstructive airway disease. However, the authors should be aware that, at least in animals, work from my laboratory has shown that activation of central 5-HT$_{1A}$ receptors causes an increase in vagal tone to the airways, producing bronchoconstriction. Furthermore, reflex activation of these bronchoconstrictor preganglionic vagal motor neurons by the stimulation of bronchial and pulmonary C-fibre ‘irritant’ receptors, can be attenuated by the central administration of 5-HT$_{1A}$ receptor antagonists and potentiated by the 5-HT uptake inhibitor fluoxetine. This indicates that a central 5-HT pathway is involved in reflex activation of bronchoconstrictor preganglionic vagal motor neurons. Furthermore, in humans, inhibition of monoamine oxidase has been shown to potentiate stimulation of airway ‘irritant’ receptors by capsaicin-induced reflex bronchoconstriction. In addition, increases in vagal tone to the airways have been shown to play an important role in nocturnal asthma. Thus, interfering with central vagal drive to the airways by blocking central 5-HT$_{1A}$ receptors might prove to be a useful adjunct to the therapy of airway disease.

The central 5-HT pathway that involves activation of 5-HT$_{1A}$ receptors might in fact be a generalized system that is involved in the reflex activation of parasympathetic preganglionic neurones to the heart, ciliary muscle and bladder as well as to the airways. In the case of the bladder, the ability to block 5-HT$_{1A}$ receptors might be useful in the treatment of hyperactive bladder. A possible unwanted effect of these antagonists is that they might cause blurred vision as a result of interference with accommodation. The fact that activation of central 5-HT$_{1A}$ receptors causes excitation, rather than inhibition, is probably a result of inhibition of a tonic GABA-mediated pathway to these neurones, which acts as a ‘brake’ on the activity of parasympathetic preganglionic neurones.

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