

and α_2 -adrenoceptor blocking properties that yield a selective increase in dopamine release in the medial prefrontal cortex represents a major challenge to the classical dopamine hypothesis of schizophrenia and their results are, at the same time, both intriguing and very interesting. Is schizophrenia in fact a frontal cortex dopamine-deficiency disorder or a disorder that involves, at the same time, hyperactive or hyperreactive subcortical dopamine systems and a hypoactive or deficient cortical dopamine system?⁷

As yet, there is very little clinical evidence to support this assumption. However, in a recent study⁸ we demonstrated, using PET, an increased dopamine synthesis rate in the medial prefrontal cortex of drug-naïve 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 neurones within the prefrontal cortex in schizophrenia, a hypothesis that was originally put forward by Weinberger *et al.*⁹ If this hypothesis turns out to be verified by further clinical evidence, the study by Hertel *et al.* might have deep implications for the pharmaceutical industry when synthesizing new antipsychotic drugs.

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Maximal function of minimal K⁺ channel subunits

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A recent report by Schroeder *et al.*¹ 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. These 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 as interesting as the associated nomenclature is confusing.

MinK (KCNE1) is a β -subunit

In 1988, Takumi *et al.* used the *Xenopus* oocyte expression system to clone a novel gene that encoded a small K⁺-channel protein². This protein was named IsK because it induced a very slowly activating outward K⁺ current when expressed in oocytes. IsK is more commonly called minK because it is the smallest protein (only 130 amino acids with a single transmembrane domain) known to be associated with K⁺-chan-

nel function. It was not until 1996 that minK was shown to co-assemble with another subunit (KvLQT1) to form the K_{V(s)} delayed-rectifier K⁺ channel^{3,4}. 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 arrhythmia⁵. KvLQT1 is a typical K⁺ channel α -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_{V(s)}]. 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 KCNE1 and its gene is *KCNE1*. KvLQT1 is now called *KCNQ1*, and three other members in this gene family have been described (*KCNQ2–4*). Mutations in any of these genes cause

human disease. For example, long-QT syndrome is caused by mutations in *KCNQ1* (or *KCNE1*)⁵. The gene products of *KCNQ2* and *KCNQ3* co-assemble to form neural M-current channels (K_M)⁶, and mutation in either gene causes benign familial neonatal convulsions^{7–9}. Finally, mutations in *KCNQ4*, which also encodes an M-current-like channel, cause neural deafness¹⁰.

KCNE1 and related proteins modulate KCNQ channel gating

In 1999, new members of the KCNE subunit family were described by Abbott *et al.*¹¹ who searched a database for expressed sequence tags for proteins with partial homology to KCNE1. Three new cDNAs related to *KCNE1* were eventually isolated using reverse transcriptase-polymerase chain reaction (RT-PCR), and the encoded proteins were named minK-related peptides (MiRP) 1, 2 and 3. The genes for the three MiRPs were named *KCNE2–4*. *KCNE2* co-assembles with an α -subunit human *ether-à-go-go-related* gene (HERG), to form the rapid delayed-rectifier K⁺ channel [K_{V(t)}]¹¹. Heterologously expressed *KCNE1* can also associate with HERG and modulate its function¹². However, this interaction

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might not be of physiological importance because HERG subunits preferentially form complexes with KCNE2 subunits when they are co-incubated with KCNE2 and KCNE1 synthesized *in vitro*¹¹. Because it was known that mutations in either the *KCNQ1* or *HERG* α -subunit genes caused long-QT syndrome^{5,13}, it was perhaps not too surprising to find that mutations in either of the associated β -subunit genes *KCNE1* or *KCNE2* also caused this inherited arrhythmia^{11,14}.

The latest chapter in the KCNE subunit story is particularly intriguing. KCNQ1 homomeric channels conduct a relatively slow delayed-rectifier K^+ current that has strong voltage-dependent gating. KCNQ1 channels open only in response to positive depolarizations 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 channels 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 subunits 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 KCNE1 and KCNQ1 subunits interact^{15,16}. In addition to altered gating, several other experimental findings reported by Schroeder *et al.*¹ indicate that KCNE3 and KCNQ1 proteins interact in a functional manner. (1) The pharmacology of KCNQ1 channel gating was markedly altered by co-expression with KCNE3; channels were more sensitive to block by chromanol 293B and clotrimazole. (2) The surface expression 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 a colonic 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 KCNE3 and KCNQ1 subunits in native cells must await the availability of specific antibodies to the two proteins. Curiously, when co-expressed with either HERG or KCNQ4 (but not KCNQ2 or KCNQ3), KCNE3 abolished functional expression¹. The physiological relevance of these interactions is uncertain, especially in tissues where *KCNE3* is poorly expressed such as the heart and cochlea, where HERG and KCNQ4 subunits, respectively, are strongly expressed and have known functions.

Physiological role for KCNQ1–KCNE3 channels

Schroeder *et al.*¹ propose that KCNQ1–KCNE3 channels conduct the basolateral 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, electrogenic Cl^- secretion, first described in tracheal epithelium by Smith and Frizzell 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 chromanol 293B and clotrimazole at concentrations 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 tissues¹⁸, it is unlikely that KCNQ1–KCNE3 channels represent the common molecular correlate of this current.

These latest findings, which describe the effect of a KCNE1-like protein on K^+ -channel gating, emphasize that the functional roles for these minK proteins are anything but minimal. Future

experiments should focus on the biophysical characterization and structural basis of how KCNE1 and KCNE3 can have such strikingly different effects on the gating of KCNQ1 channels. In addition, it will be interesting to determine the functional role of KCNE3 in tissues where it is most prominently expressed, such as the kidney and small intestine. Given the track record of KCNE1 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.

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