Paradoxical Role of T-type Calcium Channels in Coronary Smooth Muscle

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Calcium influx via voltage-gated channels plays important roles in muscle excitation-contraction coupling, hormone and neurotransmitter stimulus-secretion coupling, regulation of gene transcription, and neuronal firing. To fulfill these diverse roles, mammals have evolved ten genes encoding voltage-gated calcium channels (Cav channels) (Figure 1). These channels also serve as important drug targets, with most therapeutically useful calcium-channel blockers targeting L-type channels, such as Cav1.2, found on cardiac or smooth muscle. These drugs (e.g., amldopine) are useful in the treatment of high blood pressure because they block Ca\(^{2+}\) influx via L-type channels into smooth muscle cells (SMC), which results in relaxation.

SMCs also express T-type calcium currents (1). The development and characterization of mibebradil (also termed Ro 40-5967), a novel antihypertensive drug with modest selectivity for T-type channels (2), supported the hypothesis that blocking T-type channels would produce vasorelaxation. Mibebradil was shown to be an effective antihypertensive drug that produced coronary and peripheral vasodilation without triggering baroreceptor reflexes (2). Although mibebradil was withdrawn from clinical use, it continues to be a useful tool in the research laboratory. For example, studies on mibebradil's actions have implicated a role for T-type channels in pain perception (3–5). However, other studies have questioned the selectivity of mibebradil, showing that the drug can also block other voltage-gated calcium channels (6), sodium channels (7), potassium channels (8–10), non-selective cation currents (11), and even volume-activated chloride currents (12). These results have cast doubt on the hypothesis that mibebradil's antihypertensive action is because of blockage of T-type channels.

In order to elucidate the physiological roles of T-type channels, Chen et al. generated mice lacking Cacna1H (one of the three known T-type channel genes), which encodes the Cav3.2 or \(\alpha_{1H}\) channel (13). In contrast to the other two T-type channel genes, which are predominantly expressed in brain, Cacna1H is more broadly expressed in peripheral tissues, including heart, liver, and kidney (14). Cav3.2 is also expressed in adrenal cortex (15), and in embryonic skeletal muscle, where it plays a key role in myoblast fusion (16). Additionally, Cav3.2 mRNA is abundantly expressed in dorsal root ganglion (DRG) neurons (18). Of historical note, low voltage-activated T-type currents were originally isolated from high voltage-activated currents by voltage-clamp analysis of DRG neurons (19–21). Subsets of DRG neurons express extremely large T-type currents, suggesting that these currents are important for sensory processing. However, the role of T-type currents on sensory processing might be quite different than that of regulating intracellular calcium concentrations, and may reflect the originally ascribed role of T-type currents as a pacemaker, capable of generating low-threshold spikes that in turn initiate Na\(^{+}\)-dependent action potentials (22). To show that Cav3.2 is necessary for the formation of T-type currents, Chen and coworkers isolated neonatal DRG neurons from Cav3.2–/– mice and recorded their calcium currents using voltage-clamp techniques. These studies showed the complete ablation of T-type current with no compensation. If T-type channels were a target for novel analgesic drugs, then one would predict that these mice would show diminished responses to painful stimuli. Unfortunately, this hypothesis was not tested. Instead, Chen and coworkers used the Cav3.2–/– mice to study coronary artery contraction (13). Their surprising conclusion was that rather than mediating contraction, T-type currents mediate relaxation of coronary smooth muscle.

In contrast to littermate controls, Cav3.2–/– mice were smaller, had malformations in the spinal column, trachea, and coronary arteries, and developed cardiac fibrosis (13, 23). Surprisingly, following preconstriction with the thromboxane analog U46619, treatment with acetylcholine caused further constriction of isolated coronary arteries from Cav3.2–/– mice rather than the dilation observed in similarly treated control arteries. In addition, coronary arteries from Cav3.2–/– mice were less sensitive to the vasodilatory effects of the nitric oxide donor, sodium nitroprusside. The connection between T-type channels and nitric oxide mediated relaxation remains unclear. Perhaps gene array experiments would be useful to identify whether the expressions of other components of this pathway are affected.

Chen and coworkers focused on the possible association of Cav3.2 and calcium-activated potassium channels (BKCa), showing that both channels sediment to the bottom of a sucrose gradient, and that antibodies directed against Cav3.2 are capable of precipitating a protein recognized by antibodies directed against BKCa (13). If Ca\(^{2+}\) influxes via T-type channels are an important regulator of BKCa channels, then there may be less activation of BKCa channels in SMCs from Cav3.2–/– mice. However, similar responses were observed in normal and in Cav3.2–/– mice to the BKCa agonist NS-1619 (13). Although neuronal T-type currents have been implicated in the activation of calcium-activated K\(^{+}\) channels, these channels were voltage-independent SKCa channels (24). In contrast, neuronal BKCa channels are thought to be activated by Ca\(^{2+}\) influx via high voltage-activated Ca\(^{2+}\) channels (25). Clearly, more work is needed to establish a functional role for the association of Cav3.2 and BKCa channels.

Studies on transgenic Cav3.2–/– mice have provided definitive evidence that T-type channels produce low threshold Ca\(^{2+}\) spikes that control burst firing of thalamic neurons, and have provided insights into the role of burst firing in sensory gating (5, 26). Perhaps future studies on Cav3.2–/– mice will provide meaningful insights into the role of these channels in pain, adrenal hormone secretion, and neuronal signaling.
that obtained using membrane spanning regions. This family tree is based on the full-length human sequences, and differs from deduced amino acid sequences indicates that these channels can be grouped.

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L-type currents, and play critical roles in muscle excitation-contraction coupling. They are also the target of traditional “calcium channel blockers.” The Cav2 channels are a second subfamily of high voltage–activated channels (HVA), and they play a dominant role in neurotransmitter release. Although subtype-selective small-molecule drugs have not been developed for Cav2 channels, these channels can be distinguished with peptide toxins. The Cav3 channels carry T-type currents. These channels open after small depolarizations of the plasma membrane, and hence are also termed low voltage–acti-

vated (LVA) channels. This property allows Cav3 channels to act as a pace-
maker current, such as that originally observed in low threshold spiking and rebound burst firing of neurons.

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The serotonin-producing neurons of the brainstem raphe nuclei are a component of the reticular formation and form two main clusters of nuclei which innervate the midbrain and forebrain and the pons, medulla, and spinal cord. The superior raphe group consists of four main nuclei (the largest being the dorsal raphe nucleus (B7and B6)), and the inferior group consists of five main nuclei (the largest being the raphe magnus and raphe pallidus). Carbon dioxide chemoreceptors on medullary raphe serotoninergic neurons have been identified in in vitro preparations (1) and in whole animals (2). Recent characterization of dorsal raphe serotoninergic neurons in the midbrain has revealed that they also are chemosensitive (3). Somewhat surprisingly, the authors suggest their results are relevant to sudden infant death syndrome (SIDS), panic disorder, and migraine headaches. Severson et al. (3) state that “despite a tendency to study these neurons in relation to only a shared brain function or disease, their highly divergent projections and the homogeneity of their cellular properties ([4]) suggest that there may be a shared function of serotonergic neurons.” What is this “shared function” with respect to chemoreceptivity to CO2 and pH? One is puzzled by this cognitive leap from studies of a handful of raphe neurons in the medulla and midbrain to the whole serotoninergic system. Even if one allows for a homogenous anatomical and pharmacological classification, to invoke a “shared function” may appear overly simplistic and grandiose. The number of functions associated with serotonin (5-HT) is nearly endless. Can they all be linked?

Several authors have tried before to find this shared function. Brodie and Shore proposed a metabolic role for 5-HT in the neuronal activity of the brain (5). In their hypothesis, norepinephrine and 5-HT modulated opposite systems in the brain, based on Hess’s concept of the functional integration of the autonomic system with the CNS (6). 5-HT, it was argued, is the modulator of the trophotrophic system. As the name implies, trophotrophic is the literally the nutrition for the nutritional network, or, on an organismic level, those behavioral patterns that are recuperative in nature, such as sleep, hibernation, or eating. Later, Woolley proposed that 5-HT is essential for normal mental health (7). This hypothesis was based on the structure of 5-HT, which is similar to that of lysergic acid diethylamide (LSD), and is discussed elsewhere in a historical chapter (8). In 1975, Scheibel et al., using Golgi-stained brainstem material, noted a close relationship between the raphe reticular neurons and blood vessels (9). They proposed the raphe neurons function either as a chemoreceptor or a mechanoreceptor, although a neuroscretory role of a bioactive substance into the vascular system from these raphe neurons could not be ruled out.

If a shared function could be agreed upon, how would this finding impact therapeutic pharmacological interventions to ameliorate 5-HT neuronal dysfunction? I will first discuss the results reported by Severson et al. (3) and emphasize the earlier work performed by Scheibel et al. (9), then consider if such a shared function makes evolutionary sense. Finally, I will deal with one shared function—a mechanism of action—of serotoninergic neurons suitable for such a Herculean task, namely homeostasis.

Severson et al. (3) made perforated–patch clamp recordings from neurons in rat midbrain slices while pCO₂ was changed between 9% and 3% (pH 7.18 to pH 7.58). Out of 100 recordings, sixteen were acidosis-stimulated in the dorsal raphe nucleus and satisfied the criteria of being chemosensitive. Studies of midbrain raphe cultures showed out of seventy-eight neurons studied for their response to acidosis, twenty-one neurons were acidosis-stimulated, with an increase in firing rate to 405 ± 70% above that from control-treated neurons, in response to a decrease in pH from 7.4 to 7.2. The neurons were identified by location (in the case of the slices), firing characteristics, and in a selected number of cases by immunocytochemical staining with a specific antibody against tryptophan hydroxylase (TPH). In subsequent experiments, the authors demonstrated that major branches of the basilar artery outline the dorsal and median raphe nuclei. The arteries were visu-