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Technology Review

Atomic Absorption Spectroscopy in Ion Channel Screening

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Abstract: This article examines the utility of atomic absorption spectroscopy, in conjunction with cold flux assays, to ion channel screening. The multiplicity of ion channels that can be interrogated using cold flux assays and atomic absorption spectroscopy is summarized. The importance of atomic absorption spectroscopy as a screening tool is further elaborated upon by providing examples of the relevance of ion channels to various physiological processes and targeted diseases.

Towards Ion Channel Drug Discovery and Development

Recent years have seen a significant shift of resources in the pharmaceutical industry towards the area of ion channel drug discovery and development. This shift was compelled by the issue of drug-induced QT prolongation and the emergence of the S7B, E14 guidance documents for drug safety assessment. The importance of the hERG ion channel to drug development created significant interest in other ion channel targets. As a result, over 6 billion dollars in yearly sales and 15% of the top-selling drugs are targeted for ion channels.[1–3]

Physiological Significance of Ion Channels

Ion channels are integral membrane proteins that permit the flow of ions, such as calcium, potassium, sodium, and chloride, into and out of cells. Ion channels are present in all human cells and are involved in functions such as nerve transmission, cellular homeostasis, hormonal secretion, and the heartbeat. The wide range of physiological processes in which ion channels are involved is suggested by Table 1.

Channelopathies

With ion channels playing such an important role in many physiological functions, it is not surprising that ion channel dysfunction has been implicated in a number of diseases and disorders. Diseases that are caused by defective ion channel proteins are termed "channelopathies." Indeed, ion channels represent one of today's more promising and exciting classes of therapeutic targets on account of the broad range of conditions that are poised to benefit from agents that modulate ion channel activity. Table 2 lists some of the diseases and disorders in which ion channel dysfunction has been implicated.

Flux Assays in Ion Channel Screening

A number of technologies are available to screen against ion channel targets. Xu *et al.*[46] segmented the ion channel assay market and identified the advantages and disadvantages of various technologies. Available technologies include manual patch-clamp, automated patch-clamp instruments, fluorescence-based platforms, membrane binding assays, radioactive ionic flux assays, and non-radioactive flux assays. However, this review

Aurora Biomed Inc., Vancouver, BC, Canada.

ABBREVIATIONS: CFTR, cystic fibrosis transmembrane regulator; hERG, human *ether-a-go-go*-related gene.

Table 1. Physiological Processes Involving Ion Channels

Physiological process	*Ion channel or gene encoding ion channel*
Apoptosis	MAC[4]
	Purinergic P2X[5]
Bicarbonate secretion	SLC26[6]
Bone resorption	CIC-7[7]
	RyR[8]
Cardiac repolarization	KCNQ1, hERG, SCN5A, KCNE1, KCNE2[9]
Chloride absorption	CFTR[10]
Inflammation	ASIC's[11]
Insulin secretion	K_{ATP} (Kir6.2 and SUR1 subunits)[12]
Metastasis	CLCA[13]
Neuronal excitability	KCNQ2/KCNQ3[14]
	KCNQ5[15]
	T-type Ca channels[16]
Neuronal migration	Kv4.3[17]
Nociception	L-type Ca channels[18]
Regulation of basal metabolic rate	Kv1.3[19]
Smooth muscle relaxation	
Cavernosal	BK_{Ca}[20]
Urinary	SK_{Ca}, BK_{Ca}, and RyR[21]
Vascular	KCNMB1[22]
Sperm motility	PKD2[23]
T cell activation	Kv1.1, Kv1.3[24]

Table 2. Diseases or Disorders Involving Ion Channels

Disease or disorder	*Ion channel or gene encoding ion channel*
Andersen-Tawil's syndrome	KCNJ2[25]
Bartter's syndrome	SLC12A2, KCNJ1, ROMK, CIC-Ka, CICKb[26]
Benign familial neonatal convulsions	KCNQ2, KCN3[14]
	SCN2A[27]
Brody's disease	SERCA1[28]
Brugada's syndrome	SCN5A[29]
Periodic paralysis	SCN4A[30,31]
Cancer	CLCA[13]
Cystic fibrosis	CFTR[10]
	SLC26[6]
Epilepsy	T-type Ca channels[16]
Erectile dysfunction	BK_{Ca}[20]
Familial hemiplegic migraine	CACNA1A[32]
Heart failure	Cardiac P2X4[33]
Hypertension	KCNMB1[22]
Isolated cardiac conduction disease	SCN5A[34]
Incontinence	SK_{Ca}, BK_{Ca}, and RyR[21]
Inflammation	ASIC's[11]
Lenegre's disease	SCN5A[35]
Long-QT syndrome	KCNQ1, hERG, SCN5A, KCNE1, KCNE2[9]
	HCN4[36]
Male sterility	PKD2[23]
Multiple sclerosis	Kv1.3[37]
	Nav1.2, Nav1.6, Na^+/Ca^{2+} exchanger[38]
Myotonia congenita	CLCN1[39,40]
Myotonic dystrophy	SERCA1[28]
Neuropathic pain	Nav1.8[41]
	Nav1.9[42]
Obesity	Kv1.3[19]
Osteopetrosis	CIC-7[7]
Polycystic kidney disease	PKD2[43]
Schizophrenia	SK3[44]
Type 1 diabetes	K_{ATP} (Kir6.2 subunit)[45]
Type 2 diabetes	K_{ATP} (Kir6.2 and SUR1 subunits)[12]

TABLE 3. CHANNELS THAT HAVE BEEN EXAMINED USING THE COLD FLUX ASSAY APPROACH

Family	*Ion channel or gene encoding ion channel*
K	hERG[49,50]
	KCNQ2[51]
	BK_{Ca}[48,52]
	SK_{Ca}[48]
	Kv1.1, Kv1.4[48]
	Kv1.3[53]
Na	SCN5A[54]
Cl	CFTR[55]
Ca	Cardiac L-type[56]
Non-selective cation	Nicotinic acetylcholine[48]
	Purinergic P2X[48]
Transporter	Na^+/K^+ pump[57]
	K^+-Cl^- co-transporter[58]

will focus on the non-radioactive (cold) flux assay technology from Aurora Biomed Inc. (Vancouver, BC, Canada).

Non-radioactive flux assay technology utilizes highly sensitive flame atomic absorption spectrometers capable of dealing with the low sample volumes that are typically used in pharmaceutical research. Cold flux assays use tracer ions: molecules that are similar in size and charge to the ion of interest. To study potassium and sodium channels, the rubidium and lithium ions, respectively, are used. For chloride channels, the chloride ion itself is used with an indirect detection procedure involving precipitation with silver nitrate and measurement of the concentration of free silver.

The use of flux assays in high-throughput screening of ion channels for pharmaceutical research has been well summarized by Gill *et al.*[47] The cold flux assay as applied to the study of ion channel function is a relatively novel approach and was first proposed by Terstappen.[48] Since then, the use of cold flux assays in ion channel screening has steadily increased, and publications describing the various channels examined are listed in Table 3.

Hot flux (radioactive) assays, on the other hand, have been used for many years to assess ion channel function. Hot flux assays employ radiotracers to monitor ion flux through channels. Some of the ion channels that have been examined using radiotracers are listed in Table 4. Overall, Tables 3 and 4 represent represent some of the most recent and significant literature on their respective topics.

Any of the channels studied using the hot flux assay approach should, in theory, be amenable to the cold flux assay approach. The cold flux assays that have been developed for the Ion Channel Readers by the Aurora Biomed team include the Rb flux assay against the hERG[50] and Kv1.3[56] channels as well as against the Na^+/K^+ pump[57] and K^+-Cl^- co-transporter[58]; the Li flux assay against the SCN5A channel[53]; and the Cl flux assay for the CFTR channel.[54] The multiplicity of ion channels that

TABLE 4. CHANNELS THAT HAVE BEEN EXAMINED USING THE HOT FLUX ASSAY APPROACH

Family	*Ion channel or gene encoding ion channel*	*Radiotracer*
K	hERG[59]	$^{86}Rb^+$
	BK_{Ca}[60]	$^{86}Rb^+$
	IK_{Ca}[61,62]	$^{86}Rb^+$
	SK_{Ca}[63]	$^{86}Rb^+$
	Kv[63]	$^{86}Rb^+$
	KCh[63]	$^{86}Rb^+$
Na	Neuronal Na_v[64]	$^{22}Na^+$
Cl	CFTR[65]	$^{125}I^-$
Ca	Neuronal Ca_v[66]	$^{45}Ca^{2+}$
Non-selective cation	Nicotinic acetylcholine[67,68]	$^{86}Rb^+$
	Purinergic P2X[69]	$^{86}Rb^+$
Transporter	Na^+/K^+ pump[70]	$^{86}Rb^+$
	K^+-Cl^- co-transporter[71,72]	$^{86}Rb^+$
	Na^+-K^--Cl^- co-transporter[73,74]	$^{86}Rb^+$, $^{125}I^-$

can be examined using the cold flux assay approach is indicative of the versatility of this technique for ion channel screening.

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