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Potential role of cardiac chloride channels and transporters as novel therapeutic targets

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ABSTRACT

The heart and blood vessels express a range of anion currents (e.g. $I_{Cl.PKA}$) and symporter/antiporters (e.g. Cl^-/HCO_3^- exchanger) that translocate chloride (Cl^-). They have been proposed to contribute to a variety of physiological processes including cellular excitability, cell volume homeostasis and apoptosis. Additionally there is evidence that Cl^- currents or transporters may play a role in cardiac pathophysiology. Arrhythmogenesis, the process of cardiac ischaemic preconditioning, and the adaptive remodelling process in myocardial hypertrophy and heart failure have all been linked to such channels or transporters. We have explored the possibility that selective targeting of one or more of these may provide benefit in cardiovascular disease. Existing evidence points to an emerging role of cardiac cell anion channels as potential therapeutic targets, the 'disease-specificity' of which may represent a substantial improvement on current targets. However, the limitations of current techniques hitherto applied (such as developmental compensation in gene-modified animals) and pharmacological agents (which do not at present possess sufficient selectivity for the adequate probing of function) have thus far hindered translation to the introduction of new therapy.

Contents

Abbreviations: AE, anion exchanger; ATP, adenosine triphosphate; APD, action potential duration; cAMP, cyclic adenosine monophosphate; CA, carbonic anhydrase; CAST, Cardiac Arrhythmia Suppression Trial; CFTR, cystic fibrosis transmembrane conductance regulator; DAD, delayed afterdepolarisation; DIDS, 4,4-diisothiocyanatostilbene-2,2-disulfonic acid; DNDS, 4,4′-dinitrostilbene-2,2′-disulphonic acid; E_{Cl}, reversal potential for chloride; ECG, electrocardiogram; Ix, current carried by the ion(s) x; IPC, ischaemic preconditioning; mRNA, messenger ribonucleic acid; NBD(x), nucleotide binding domain (x); PKA, protein kinase A; PKC, protein kinase C; QT_{90}, QT interval at the point of 90% repolarisation; RT-PCR, reverse transcription polymerase chain reaction; SITS, 4-acetamido-4-isothyocyanatostilbene-2,2-disulfonic acid.

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1. Introduction

In 1961, electrophysiological studies of cardiac cell anion channels by Hutter and Noble, and Carmeliet demonstrated that replacement of extracellular Cl^- with an impermeable anion resulted in prolongation of ventricular action potential duration (APD and effects on maximum diastolic potential (Carmeliet, 1961); Hutter & Noble, 1961). Although subsequent investigations in the late 1960s and early 1970s suggested the existence of a cardiac transient outward current carried by Cl^- (Dudel et al., 1967; Fozzard et al., 1973), the ensuing discovery that K^+ was largely responsible for '*the*' 'transient outward current' in sheep Purkinje fibres (Kenyon & Gibbons, 1979a,b) meant that the role of anions in cardiac cell electrophysiology was hereafter, for many

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years, largely ignored, with cardiac physiological and pharmacological research focusing on cation (K^+, Na^+ and Ca^{2+}) channels (Sanguinetti & Bennett, 2003). However, the relatively more recent discovery of the existence of several types of Cl^- currents (Bahinski et al., 1989; Harvey & Hume, 1989) and the molecular, genetic and biophysical characterisations of many of the channels that carry such currents (Table 1), have initiated a renewed, though nevertheless episodic, interest in the functions played by cardiac Cl^- currents in normal and abnormal electrophysiology, drug discovery (Curtis et al., 1993; Diaz et al., 2010) and drug safety (Kominkova et al., 2013).

It is now known that a variety of Cl^- channels in the mammalian heart may contribute to a host of physiological cellular functions (including cellular excitability, cell volume homeostasis and apoptosis), and that they may play an important role in arrhythmogenesis, the process of cardiac ischaemic preconditioning, and the adaptive remodelling process in myocardial hypertrophy and heart failure (Duan et al., 2005). However, this has yet to lead to translation — the introduction of new therapy. In this review, we have considered the physiological properties of the known Cl^- channels, as well as the electroneutral anion pumps and cotransporters maintaining cellular Cl^- homeostasis, and have evaluated the emerging prospect of these molecular entities as targets for the discovery of therapeutic agents.

One factor confounds interpretation of literature in this field. There are sometimes conflicts between apparent effects of target-specific drugs and apparent absence of target expression. The most likely explanation is that the target-specific drugs used are not target selective. Table 1 summarizes key data, much of it derived from the Concise Guide to Pharmacology (Alexander et al., 2013). The table leaves gaps where knowledge is speculative or absent.

2. Electroneutral anion transporters and cotransporters

The estimated intracellular Cl^- activity in Purkinje fibres and myocardial ventricular cells of 10–20 mM (e.g. Baumgarten & Fozzard, 1981) is much higher than that predicted from the Nernst equation if Cl^- were distributed only by passive diffusion (4–6 mM; Hume et al., 2000), and estimates become even higher during ischaemia (Ramasamy et al., 1992). This apparent intracellular accumulation of Cl^- into myocardial ventricular cells occurs through a variety of electroneutral anion transporters and cotransporters, achieving a reversal potential for Cl^- (E_{Cl}) in the heart of between −40 and −60 mV (Hume et al., 2000).

Among the known transport proteins in the mammalian heart, the Cl^-/HCO_3^- exchanger is the most extensively characterised. It has various isoforms encoded by the *Slc4a* gene family (AE1, AE2 and AE3) (Kopito, 1990), and members of the *Slc26* gene family (Everett & Green, 1999; Alvarez et al., 2004), with *Slc26a6* having been cloned from human myocardium (Lohi et al., 2000; Waldegger et al., 2001). The isoforms have a dual function as Cl^-/HCO_3^- and Cl^-/OH^- exchangers, being highly expressed in cardiac tissues and having particular potential importance in the regulation of myocardial intracellular pH (Alvarez et al., 2004). Of note, it has been found that α_1-adrenergic agonism and protein kinase C (PKC) stimulation attenuate *Slc26a6* Cl^-/HCO_3^- exchange activity, whereas PKC stimulation induces the opposite response in the AE3 exchanger. On this basis it has been suggested that *Slc26a6* may play a role in limiting the progression of myocardial hypertrophy associated with sustained adrenergic drive to the heart (Alvarez et al., 2004).

Other transport proteins include the Na^+–Cl^-, Na^+–K^+–$2Cl^-$, and K^+–Cl^- cotransporters, with the probable contribution of a Cl^-/OH^- exchanger (CHE) contributing towards maintenance of myocardial intracellular pH (Sun et al., 1996; Hun Leem & Vaughan-Jones, 1997) as has been demonstrated in guinea-pig ventricular myocytes (Niederer et al., 2008). Na^+–Cl^- and K^+–Cl^- cotransporters are inevitably dependent on Na^+ and K^+ gradients respectively, maintained by the Na^+–K^+ pump (Hume et al., 2000).

In addition to counteracting passive Cl^- efflux and electrogenic Cl^- movement, the aforementioned anion transporters and cotransporters contribute to cell volume homeostasis (Hume et al., 2000). Additionally, the Cl^-/HCO_3^- exchanger plays an important role in the maintenance of intracellular pH through myocyte acid loading, part of the heart's evolved complex system for handling metabolic fluctuations in acid or base, and thus maintaining cardiomyocyte intracellular pH at ~7.2 (Vaughan-Jones et al., 2009). In regulating intracellular pH, such anion

Table 1
Summary of key characterisation data from Concise Guide to Pharmacology (Alexander et al., 2013) and other papers cited in this review.

Name	Abbreviation	Type	Gene encoding	Inhibitor	Activator	In heart?	Primary cardiac effect of antagonism
Volume regulated anion channel	VRAC	Channel	Uncertain	Tamoxifen	GTPγS	Unknown	Selectivity issues preclude certainty
Maxi chloride channels	Maxi Cl	Channel	Uncertain	DIDS	Tamoxifen	Yes	Selectivity issues preclude certainty
Calcium activated chloride channel	CaCC	Channel	ANO1	Crofelemer	Intracellular ca	Unlikely	Selectivity issues preclude certainty
Cystic fibrosis transmembrane conductance regulator	CFTR	ABC class transporter	CFTR	Crofelemer	Felodipine	Yes	Selectivity issues preclude certainty
Chloride channel 1	ClC-1	Channel	CLCN1	Niflumic acid	None known	Unknown	Unknown
Chloride channel 2	ClC-2	Channel	CLCN2	Natriuretic peptide B	Omeprazole	Yes	Unknown
Chloride channel Ka	ClC-Ka	Channel	CLCNKA	DIDS	Niflumic acid	Unknown	Unknown
Chloride channel Kb	ClC-Kb	Channel	CLCNKB	DIDS	Niflumic acid	Unknown	Unknown
Chloride channel 3	ClC-3	Cl/H antiporter	CLCN3	Phloretin	Unknown	Unknown	Unknown
Chloride channel 4	ClC-4	2Cl/H antiporter	CLCN4	Zinc ion	Unknown	Unknown	Unknown
Chloride channel 5	ClC-5	2Cl/H antiporter	CLCN5	*Not* DIDS/niflumic acid	Unknown	Unknown	Unknown
Chloride channel 6	ClC-6	2Cl/H antiporter	CLCN6	DIDS	Unknown	Unknown	Unknown
Chloride channel 7	ClC-7	2Cl/H antiporter	CLCN7	DIDS	Unknown	Unknown	Unknown
Anion exchange protein 1	AE1	Cl/HCO3 antiporter	SLC4A1	Unknown	Unknown	Probably	Selectivity issues preclude certainty
Anion exchange protein 2	AE2	Cl/HCO3 antiporter	SLC4A2	Unknown	Unknown	Probably	Selectivity issues preclude certainty
Anion exchange protein 3	AE3	Cl/HCO3 antiporter	SLC4A3	Unknown	Unknown	Probably	Selectivity issues preclude certainty
Na-driven Cl-HCO_3 exchanger	NBCBE	Cl/Na:2HCO3 antiporter	SLC4A8	Unknown	Unknown	Unknown	Unknown
Bicarbonate transporter-related protein	NaBC1	Cl/HCO3 antiporter	SLC4A11	Unknown	Unknown	Unknown	Unknown

Note that there are numerous specific activators and inhibitors of most of these targets. However the selectivity is questionable in almost all instances. One (more common) example of each is shown, if identified.