British Journal of Pharmacology (2008) 153, 844–845
 0007–1188/08 $30.00
www.brjpharmacol.org

# COMMENTARY

# Blowing off acid: a new tool to study $Na^+/HCO_3^-$ co-transport

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Investigation of the physiological functions and possible pathological roles of $Na^+/HCO_3^-$ co-transport in the heart has been hampered by uncertainty over the molecular identity of cardiac $Na^+/HCO_3^-$ co-transporter(s) and the absence of selective pharmacological inhibitors. In their paper published in this issue, Ch'en and colleagues describe the extensive characterization of S0859 as a high-affinity inhibitor of $Na^+/HCO_3^-$ co-transport in cardiac myocytes (Ch'en *et al.*, 2008). The availability of S0859 provides a powerful new tool to investigate the (patho)physiological significance of $Na^+/HCO_3^-$ co-transport in the heart and other tissues.
*British Journal of Pharmacology* (2008) **153,** 844–845; doi:10.1038/sj.bjp.0707670; published online 21 January 2008

**Keywords:** pH regulation; heart; $Na^+/HCO_3^-$ co-transport; $Na^+/H^+$ exchange; ischaemia

**Abbreviations:** NBC, $Na^+/HCO_3^-$ co-transporter; NHE, $Na^+/H^+$ exchanger; $pH_i$, intracellular pH

In cardiac myocytes, as in other cell types, numerous cellular processes are sensitive to changes in intracellular pH ($pH_i$). In particular, almost every step in the excitation–contraction process in cardiac myocytes is inhibited by intracellular acidosis (Orchard and Kentish, 1990), emphasizing the importance of $pH_i$ regulatory mechanisms that have evolved to prevent or correct this condition. In these highly differentiated cells, $pH_i$ regulation is accomplished through sarcolemmal acid/base transport proteins and intracellular buffers working in concert, with the diffusive movement of protons coupling the two processes (Vaughan-Jones *et al.*, 2006a).

In cardiac myocytes, the trans-sarcolemmal extrusion of acid equivalents in response to an intracellular acid challenge is accomplished primarily by $Na^+/H^+$ exchanger (NHE) and $Na^+/HCO_3^-$ co-transporter (NBC) proteins (Leem *et al.*, 1999); the pertinent ion transport processes are driven by the inwardly directed $Na^+$ gradient across the sarcolemma and import $Na^+$ ions while exporting $H^+$ or importing $HCO_3^-$ (Vaughan-Jones *et al.*, 2006b). The molecular identity of the cardiac sarcolemmal NHE is well-established as NHE1, which is the protein product of the *SLC9A1* gene (Orlowski and Grinstein, 2004), and several NHE1-selective pharmacological inhibitors such as cariporide, eniporide, sabiporide and zoniporide have been developed and characterized. In contrast, the protein products of multiple members of the *SLC4* gene family (such as *SLC4A4*, *SLC4A5* and *SLC4A7*) (Romero *et al.*, 2004) are likely to contribute to $Na^+/HCO_3^-$ co-transport activity in cardiac myocytes, and selective inhibitors of the pertinent NBC isoforms have not yet been described.

In a further addition to the fundamental contributions from the Vaughan-Jones laboratory to the understanding of $pH_i$ regulatory mechanisms in cardiac myocytes, the characterization of a high-affinity inhibitor of $Na^+/HCO_3^-$ co-transport in cardiac myocytes, in the form of the *N*-cyanosulphonamide compound S0859, is presented in this issue of the *British Journal of Pharmacology* (Ch'en *et al.*, 2008). Interestingly, S0859 appears to inhibit all active NBC moieties in cardiac myocytes, regardless of their molecular identities, without affecting NHE1 activity. As such, S0859 should now provide a powerful new tool in delineating the relative contributions of myocardial acid extrusion via NHE1 versus NBCs in various physiological and pathological settings, particularly when used in conjunction with NHE1-selective inhibitors. In this context, NHE1-selective pharmacological inhibitors have been very valuable in determining the role of $Na^+/H^+$ exchange in the pathogenesis of cardiac injury and dysfunction during ischaemia and reperfusion, in animal models and human studies (Avkiran and Marber, 2002). Interestingly, although NHE1 activity appears to be the major $Na^+$ influx mechanism underlying intracellular $Na^+$ accumulation during myocardial ischaemia (Hartmann and Decking, 1999; Ten Hove *et al.*, 2005), studies in isolated hearts perfused with $HCO_3^-$-free medium suggest that NBC activity also makes an important contribution (Ten Hove *et al.*, 2005). The use of S0859 in appropriately designed studies should facilitate consolidation of the role of NBC activity in the disruption of intracellular

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Received 26 November 2007; accepted 5 December 2007; published online 21 January 2008

$Na^+$ (and consequently $Ca^{2+}$) homeostasis during ischaemia, and help assess the potential of NBC inhibition as a novel therapeutic approach in myocardial ischaemia. There is also evidence that electrogenic $Na^+/HCO_3^-$ co-transport in myocardium may regulate resting membrane potential and action potential duration (Villa-Abrille *et al.*, 2007) and that depolarization-induced activation of such a process may contribute to acid extrusion from myocardium following an increase in beating rate (Camilion de Hurtado *et al.*, 1996). Again, S0859 may prove a useful tool in substantiating these hypotheses. Finally, there appears to be increased NBC expression and activity in rat myocardium, following pressure overload-induced hypertrophy (Yamamoto *et al.*, 2007). The use of S0859 to inhibit NBC activity may help evaluate the functional importance of such a change and reveal whether it is compensatory or detrimental in nature.

The pertinent work of Ch'en *et al.* (2008) does not allow any conclusions to be drawn on the identities of the *SLC4* gene family members, whose protein products contribute to NBC activity in cardiac myocytes, or on the presence or absence of any isoform selectivity in the inhibitory effects of S0859. Nevertheless, as S0859 appears to be a generic inhibitor of NBC activity, regardless of the molecular identity of the underlying transporter proteins (Ch'en *et al.*, 2008), its use should allow determination of the physiological and pathological roles of NBC activity in myocardium. Once this is accomplished, further work will be required to determine the roles of individual NBC isoforms. Recent advances in molecular techniques to suppress the expression of specific genes in cardiac myocytes, for example, through the adenoviral delivery of short-hairpin RNA moieties targeted at the selected mRNAs (Cuello *et al.*, 2007), should facilitate the necessary progress, as a prelude to more demanding studies utilizing gene targeting in mice. With the addition of S0859 to the investigators' armoury, the NBC field is now ripe for the complementary application of pharmacological and molecular approaches in deciphering the roles of $Na^+/HCO_3^-$ co-transport in healthy and diseased myocardium, as well as in other tissues.

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