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Why did the NHE inhibitor clinical trials fail?

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Murphy

Karmazyn [1] first showed that the Na–H exchange (NHE) inhibitor amiloride improved the contractile recovery of the isolated rat heart following ischemia. This result has been repeatedly confirmed in a range of animal models and with various NHE1 inhibitors and has naturally triggered interest in whether ischemic damage to human hearts would show a similar benefit.

There has been some disagreement regarding the involvement of the plasma membrane NHE in the rise in intracellular Na⁺ ([Na⁺]_i) during ischemia [2–6]. Fig. 1 illustrates the proposed role of NHE in causing a rise in [Na⁺]_i which then stimulates a rise in cytosolic Ca²⁺ via the Na–Ca exchange. This controversy has implications for a number of recent clinical trials. The main area of disagreement seems to be whether NHE contributes to the rise in Na_i during ischemia. I would argue that the data suggest that it does. I propose that we discuss the controversy by focusing on the following questions.

1. Does [Na⁺]_i rise during ischemia?

Most data in the literature show a rise in [Na⁺]_i during ischemia in perfused hearts [6–11]. Some earlier studies by Allen and coworkers, using rat hearts paced at 2 Hz and at 35 °C, found little or no increase in [Na⁺]_i during ischemia [2,5,11]. The smaller rise in [Na⁺]_i in these studies could be related to the lower rate of beating and lower ischemic temperature. Consistent with the effect of metabolic rate on [Na⁺]_i during ischemia, a recent study by Williams et al. with ischemia at 37 °C and pacing at 5 Hz reported ~ a 10 mM increase (a doubling) in [Na⁺]_i during ischemia [6]. This increase is still somewhat lower than the typically 20–30 mM increase in [Na⁺]_i observed with ²³Na NMR [7]. Interestingly Williams et al. used female rats, and a recent study by Imahashi et al. [12] found that females had a slightly but significantly lower rise in [Na⁺]_i during ischemia. Taken together, although there may be some difference in the extent of the rise in [Na⁺]_i during ischemia depending on pacing rate, temperature, and gender, there appears to be agreement that in beating perfused hearts paced at 5 Hz at 37 °C there is an increase in [Na⁺]_i during ischemia.

2. Is NHE responsible for the rise in [Na⁺]_i during ischemia?

A number of studies have reported a rise in [Na⁺]_i during ischemia that was blocked by NHE inhibitors such as amiloride [7,8]. We interpreted these data as showing a role for NHE in [Na⁺]_i loading during ischemia. This interpretation was questioned based on the following points. Firstly, the low extracellular pH during ischemia has been suggested to inhibit NHE. Secondly, amiloride was shown to also block persistent Na⁺ channels, and these persistent Na⁺ channels will result in [Na⁺]_i entry during ischemia. Thirdly, although amiloride inhibited the rise in [Na⁺]_i during ischemia, it did not increase the fall in pH during ischemia as might

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be expected with inhibition of NHE. We have responded to these issues as follows. Although low extracellular pH will reduce the rate of NHE activity, some low level of activity can still occur [13]. Slowing the rate of a NHE is not the same as inhibition. More importantly, the fall in extracellular pH does not occur immediately and therefore NHE will operate during the beginning of ischemia leading to a rise in $[Na^+]_i$. The second point regarding the inhibition of persistent Na^+ channels by amiloride and related compounds is an issue that was not fully recognized in our original paper, in which we attributed all of the amiloride dependent inhibition of the rise in $[Na^+]_i$ during ischemia to inhibition of NHE. It is clear that amiloride can inhibit persistent Na^+ channels and therefore some of the amiloride dependent increase in $[Na^+]_i$ during ischemia is likely due to amiloride mediated inhibition of persistent Na^+ channels; we have acknowledged this point in reviews over the past 10 years [4]. Inhibition of persistent Na^+ channels by TTX and lidocaine has been shown to reduce the rise in $[Na^+]_i$ during ischemia [6,9]. However addition of lidocaine reduced but did not block the rise in $[Na^+]_i$ during ischemia [9], suggesting that other mechanisms are involved. Furthermore a role for Na^+ channels in the rise in $[Na^+]_i$ during ischemia does not preclude a role for NHE. Indeed recent studies with more specific NHE inhibitors show that these more specific inhibitors also reduce the rise in $[Na^+]_i$ during ischemia [14–16]. Also, consistent with NHE-dependent effects during ischemia, mice lacking NHE were shown to be resistant to ischemia–reperfusion injury with better preservation of ATP *during ischemia* and a reduced ischemic contracture [17]. These beneficial effects are consistent with an effect of NHE *during ischemia*. Furthermore there does not appear to be a clear relationship between inhibition of persistent Na^+ channels and inhibition of the rise in $[Na^+]_i$ during ischemia. Williams et al. [6] report that zoniporide reduced persistent Na^+ channels to 41% (a 60% reduction), but yet had no effect on the rise in $[Na^+]_i$ during ischemia. In contrast, Baetz et al. [18] report that cariporide reduced the Na^+ current by 24%, but the rise in $[Na^+]_i$ during ischemia by over 50%. If the rise in $[Na^+]_i$ during ischemia is due only to the inhibition of the persistent Na^+ channels, these results are difficult to interpret. Regarding the third point, the NHE is not the only pH regulatory transporter and although inhibition of NHE would be expected to change the rate at which pH reaches its set point, it would not necessarily alter the final pH. Also inhibition of NHE during ischemia would reduce the Ca^{2+} load during ischemia and this would reduce Ca^{2+} activation of the sarcoplasmic reticulum ATPase and thereby reduce metabolism and generation of metabolic acid. The reduction in Ca observed with NHE inhibitors would also be expected to indirectly alter pH_i by reducing $[Ca^{2+}]_i$ release of H^+ from common binding sites [19]. Thus, I would suggest that both NHE and persistent Na^+ channels contribute to the rise in $[Na^+]_i$ during ischemia.

3. Does $[Na^+]_i$ rise during reperfusion?

In perfused heart studies using ^{23}Na NMR, there is little or no measurable increase in bulk $[Na^+]_i$ on reperfusion [10,20]. However, this lack of rise in $[Na^+]_i$ on reperfusion does not mean that NHE does not result in Na^+ entry during ischemia. Indeed if the Na–K ATPase is inhibited during reperfusion there is a measurable rise in $[Na^+]_i$ on reperfusion, which is reduced in the presence of NHE inhibitors [10]. Taken together most data suggest that NHE is active on reperfusion and results in Na^+ entry into the myocytes; however the Na^+ that enters via NHE is rapidly removed from the myocyte via Na–KATPase and the Na–Ca exchanger. In contrast to the studies showing little or no rise in $[Na^+]_i$ during reperfusion, Williams et al. report a very large rise on reperfusion to levels of ~40 mM [6]. The reasons for this large rise in $[Na^+]_i$ on reperfusion are unclear and I would be interested in Dr. Allen's thoughts on this issue.

4. Is NHE responsible for the rise in $[Na^+]_i$ during reperfusion?

I think there is generally an agreement that NHE is active during reperfusion. Inhibitors of NHE slow the rate at which intracellular pH returns to normal [20]. Inhibitors of NHE will also reduce the rise in $[Na^+]_i$ during reperfusion in hearts in which the Na–K ATPase is inhibited [10].

Allen

We both agree that $[Na^+]_i$ rises during ischemia and that the critical question is the mechanism by which NHE inhibitors achieve the reduction in $[Na^+]_i$ during ischemia. We also agree that during reperfusion there is additional Na^+ entry which often causes a further transient rise in $[Na^+]_i$ e.g. Fig. 2A. Murphy et al. [7] showed that amiloride reduced the ischemic rise of $[Na^+]_i$ and this result has been confirmed by many groups e.g. Fig. 2B. Murphy et al. proposed that NHE1 had been activated by the large intracellular acidosis of ischemia and was removing H^+ in exchange for Na^+ entry. You now concede that this interpretation is challenged by (i) the large extracellular acidosis would be expected to inhibit NHE1 [21], (ii) the fact that all NHE1 inhibitors so far tested (amiloride, EIPA, cariporide and zoniporide) appear to block the persistent Na^+ channels [6,18,22,23] and (iii) although NHE inhibitors reduce the rise of $[Na^+]_i$ they do not increase the magnitude of the acidosis. A further challenge to this interpretation is the finding that metabolic inhibition [24,25], hypoxia [11] and ischemia [11] have all been shown to inhibit NHE1 although the mechanism remains uncertain [26].

An alternative (or additional) hypothesis to explain the ischemic rise in $[Na^+]_i$ is that it arises through Na^+ channels. During ischemia conventional (transient) Na^+ channels are inactivated by depolarization [27] but the activity of persistent Na^+ channels are both enhanced by hypoxia and unaffected by depolarization [28]. A number of studies have shown that the rise of $[Na^+]_i$ during ischemia can be blocked by drugs which block the persistent Na^+ channels [2, 23,29] and an example is shown in Fig. 2C.

You argue above that the problem of persistent Na^+ channel inhibition by NHE inhibitors may be minimal on the grounds that “more specific NHE inhibitors also block the rise of $[Na^+]_i$ ”. Recent NHE1 inhibitors are certainly more potent and more selective against NHE1 compared to the other NHE subtypes [30] but whether they are more specific, in the sense of relatively greater NHE1 inhibition v. side effects such as blocking persistent Na^+ channels, remains to be determined. For example, we recently tested zoniporide, which is a potent inhibitor of NHE1 [30] and found it to have substantially less persistent Na^+ channel blockade than amiloride, but, as shown in Fig. 2D, it had no effect on the rise of $[Na^+]_i$ during ischemia in contrast to your assertion.

Thus many studies support the idea that persistent Na^+ channels contribute to the rise of $[Na^+]_i$ during ischemia, and the quantitative role of NHE1 has yet to be reassessed following the discovery that many NHE1 inhibitors also block persistent Na^+ channels. In contrast there is general agreement in the field that the Na^+ entry during reperfusion is principally through the NHE1 and is consequently blocked by either amiloride or zoniporide (Figs. 2B, D) but not by a low concentration of tetrodotoxin (Fig. 2C).

An interesting feature of the literature is that in most studies e.g. [7] $[Na^+]_i$ falls monotonically on reperfusion whereas in some studies $[Na^+]_i$ rises transiently on reperfusion (e.g. Fig. 2A). Presumably $[Na^+]_i$ rises if Na^+ influx through NHE1 and other sources is greater than Na^+ extrusion by the Na^+ pump and the Na^+/Ca^{2+} exchanger. An interesting insight into this variability is provided by a study of cardiac ischemia in which Na^+ pump activity and $[Na^+]_i$ were measured in hearts during low flow ischemia [31]. When glucose was absent from the perfusate, the rise in ischemic $[Na^+]_i$ was large and the Na^+ pump was inhibited, and when these hearts were reperfused the $[Na^+]_i$ fell monotonically, suggesting that reactivation of the Na^+ pump and efflux on the Na^+/Ca^{2+} exchanger overwhelmed any Na^+ influx associated with NHE1 activity. Conversely, when glucose was present, the Na^+ pump showed continued activity during ischemia and the $[Na^+]_i$ rose minimally, and on reperfusion, $[Na^+]_i$ showed a transient rise. Thus aspects of the metabolic state dependent on the degree of ischemia influence

the rise of $[Na^+]_i$ during ischemia and reperfusion and may well influence the response to NHE1 inhibitors.

These issues are important because they inform how and when to administer an NHE1 inhibitor or a persistent Na^+ channel blocker to obtain maximal protection during an ischemic episode. It is generally agreed that the level to which $[Na^+]_i$ rises is inversely related to the recovery of function after a period of ischemia/reperfusion; thus the aim of treatment is to minimize the rise in $[Na^+]_i$. Obviously since Na^+ appears to enter during both ischemia and reperfusion it would be logical to block both paths if feasible; thus optimal therapy would include a persistent Na^+ channel blocker and an NHE1 inhibitor during the ischemic period and a NHE1 inhibitor during the reperfusion period. Thus it may yet turn out to be a clinically valuable feature that most NHE1 inhibitors also block persistent Na^+ channels.

Murphy

Given the very strong experimental support for cardioprotection by NHE inhibitors in animal models of cardiac ischemia, a key question is understanding why the clinical trials with NHE inhibitors failed to show protection.

Clinical trials to test the beneficial effects of NHE inhibitors in the setting of myocardial infarction have largely failed to show a benefit [32]. A *post hoc* analysis, of a study in which the NHE inhibitor cariporide was administered to patients undergoing coronary artery bypass (CABG), showed a beneficial effect [33]. In this study, a one-hour intravenous infusion was given before surgery and administered every 8 h for 2 to 7 days. This beneficial effect in CABG patients was attributed, at least in part, to the administration of cariporide to the CABG cohort prior to surgery (ischemia). A follow up trial to test the effect of cariporide in CABG used a higher dose of cariporide and the trial was stopped due to an increased incidence of stroke. Interestingly in this prospective trial the NHE inhibitor cariporide given prior to CABG significantly reduced myocardial infarction (MI) and MI deaths compared to the placebo group. However, this beneficial effect was offset by an overall increase in mortality from 1.5% in the placebo group to 2.2% in the cariporide group; this increase in mortality was due to an increase in cerebrovascular events [34].

The failure of the clinical trials is consistent with the animal data that show consistent protection with NHE inhibitors only when the inhibitors are present during ischemia as well as during reperfusion [1,7,35]. A number of studies comparing the effects of NHE inhibitors given during ischemia and reperfusion to NHE inhibitors given only at the start of reperfusion have found protection only when the inhibitors are present during ischemia [35]. A few studies have found partial protection when NHE inhibitors are given at the start of reperfusion [5,36]. Overall these data are consistent with a beneficial role for NHE during ischemia. It is likely that NHE inhibitors would also provide some partial benefit if they could be given immediately on reperfusion. However, even when the inhibitor is given immediately at reperfusion, it takes some time for it to reach the myocytes and if the inhibitor is not present during the first minutes of reperfusion the inhibitor is unlikely to be of much benefit.

Allen

Several human trials of NHE1 inhibitors in various types of cardiac ischemia have been published and their outcomes have recently been reviewed [37]. Generally the results have been disappointing. In two trials patients reaching hospital with acute myocardial infarcts were given i.v. NHE1 inhibitors shortly before reperfusion. In one small trial with cariporide [38] a benefit was apparent but in a larger trial with eniporide there was no significant effect [39]. Dr Murphy and Avkiran et al. [37] argue that this failure is because NHE1 inhibition is most effective during ischemia. However, as discussed above, this is not consistent with the evidence

that NHE1 is inhibited during ischemia. We have previously suggested [40] that NHE1 inhibitors may not have time to access the reperfused tissue before most Na^+ influx has occurred, [35] which from Fig. 2A appears to be within 10–20 min. An alternative strategy might be to administer the NHE1 inhibitor via the angioplasty catheter. This has several advantages; (i) the drug can be targeted to the ischemic region, (ii) the drug can be applied before significant reperfusion overcoming the access problem proposed above and (iii) the systemic dose of the drug can be minimized. While this approach has not been attempted clinically to our knowledge, an important animal study by Klein et al. [35] appears to argue forcefully against it. This study measured infarct size in a pig model following coronary artery occlusion. A low flow was maintained during ischemia to allow drug perfusion. The study showed a beneficial effect of cariporide if applied during ischemia but not if applied during reperfusion. The strengths of this study are that the cariporide concentration was monitored and since it was applied 10 min before reperfusion there is no question of inadequate access. However a low flow model will have a quite different metabolic profile from ischemia and may well activate Na^+ entry by different pathway to more complete degrees of ischemia so there is a possible concern as to whether this result is relevant to ischemia.

A second group of clinical trials were motivated by two issues. (i) The view that NHE1 activity is high during ischemia and therefore patients need to be treated during the period of ischemia. (ii) The desirability of identifying a large group of patients who will benefit from the drug. The GUARDIAN trial [32] therefore targeted several groups; (i) patients with unstable angina, (ii) high risk patients about to undergo angioplasty and (iii) patients about to undergo coronary bypass surgery. These trials were negative except that sub-group analysis suggests that highest dose of cariporide exerted some beneficial effects in the patients undergoing CABG. The value of cariporide in reducing myocardial infarcts during CABG was confirmed in the aborted EXPEDITION trial [34].

Human trials can fail for many reasons but a primary reason must be that animal models of disease on which they are based are oversimplified. Dr Murphy and Avkiran et al. [37] make the case that current trials failed because they did not target the period of ischemia which is when NHE1 inhibition is most successful. I believe their view to be incorrect in so far as NHE1 inhibition is concerned but correct for the current NHE1 inhibitors which all exhibit some degree of persistent Na^+ channel inhibition. However we should bear in mind that trials can also fail for many other reasons. The large, diverse trials [32] may have failed because very few of the subjects experienced ischemia; the post-infarct trials may have failed because of failure to target the drug to the critical site within the window of opportunity which appears to start immediately after reperfusion and to last only 10–20 min.

Murphy

Although the debate over the mechanism by which drugs such as cariporide inhibit the rise in $[\text{Na}^+]_i$ during ischemia is of basic interest (and may help design better drugs), it does not address the important question as to why the clinical trials with NHE inhibitors failed to show protection. I fully agree with Dr. Allen that optimal therapy would include a persistent Na^+ channel blocker and an NHE1 inhibitor during ischemia and an NHE inhibitor (and possibly a Na channel inhibitor) on reperfusion. However, Dr. Allen appears to disagree with his statement later when he states that the failure of the NHE1 inhibitor trials was not likely to be due to the lack of addition of NHE1 inhibitors during ischemia, because “this is not consistent with the evidence that NHE1 is inhibited during ischemia.” Regardless of the mechanism, NHE1 inhibitors have been shown to block the rise in $[\text{Na}^+]_i$ during ischemia and this reduction in ischemic $[\text{Na}^+]_i$ would not occur if the NHE1 inhibitor is not applied during ischemia. Dr. Allen suggests that the failure of the clinical trial may be because the NHE1 inhibitors do not have time to reach the myocytes before Na^+ influx has occurred. This is likely to be a

contributing factor. However the majority of the preclinical data suggest that these inhibitors show most benefit when added during ischemia and during the initial period of reperfusion. NHE1 inhibitors given only at the start of reperfusion in animal studies, where this can be better controlled than in a human clinical trial, showed that NHE1 inhibition only on reflow is not as protective as NHE1 inhibition during ischemia alone or ischemia and reperfusion. The assumption “that the clinical trials failed because they did not target the period of ischemia when NHE1 inhibition is most successful” is based on a large number of studies and the assumption is independent of the mechanism by which NHE1 inhibitors block the rise in $[Na^+]_i$ during ischemia. It is certainly possible that other factors contributed to the failure of the NHE1 clinical trials; however based on the preclinical data the timing of administration of the inhibitors would appear to be at least one likely factor.

Biographies

David Allen is Professor of Physiology at the University of Sydney and a member of the Bosch Institute for Medical Research. He trained in Physiology and Medicine at University College London where he also undertook his PhD on the activation of cardiac muscle. As a post-doctoral fellow he joined John Blinks at the Mayo Clinic in Minnesota where they devised the first method for measuring intracellular calcium in the heart. On his return to University College as a Lecturer then Reader he undertook a series of studies of calcium regulation in the heart and, in particular, its modification by muscle length and ischaemia. In 1989 he moved to Sydney where his research encompasses pacemaker regulation, ischaemia and reperfusion of the heart, skeletal muscle fatigue and the damage pathways in muscular dystrophy. He has been on the Editorial Boards of the Journal of Physiology, Circulation Research, Pflugers Archiv, Heart, Lung and Circulation and the Journal of Molecular and Cellular Cardiology. In 2006 he was made a Fellow of the Australian Academy of Science and in 2007 he was the UK/Australian Physiological Society Prize Lecturer and gave the Basic Science Lecture of the Cardiac Society of Australia and New Zealand.

Dr. Elizabeth Murphy is the Head of the Cardiac Physiology Section in the Translational Medicine Branch at the National Heart, Lung and Blood Institute. She received her PhD from the University of Pennsylvania in Biochemistry, followed by postdoctoral studies in Physiology at Duke University, before joining the National Institutes of Health. She is an Associated Editor for the *Journal of Molecular and Cellular Cardiology* and she also serves on the editorial board of *Circulation*, *Circulation Research*, and the *American Journal of Physiology*. She is a member of NIH Study Section (Electrical Signaling, Transport and Arrhythmias). She has served on a number of American Heart Association committees, and is Secretary of the American Section of the International Society for Heart Research. She has

authored over 100 peer-reviewed papers. Her research has focused on ionic and energetic alterations in cell death and cardioprotection, and the signaling pathways that control these pathways.

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Fig. 1. $[Na^+]_i$ has been shown to rise during ischemia concomitant with a fall in pH_i . The mechanism for the rise in Na during ischemia is somewhat controversial. It has been attributed to activation of NHE, activation of persistent Na channels or a combination of both.

Fig. 2. $[Na^+]_i$ measurements from isolated rat hearts (37 °C, 5 Hz stimulation rate, perfused at a constant flow rate) subjected to 30 min global ischemia (flow stopped). Drugs applied as indicated by lower bar. Modified from Williams et al. [6].