NO and the regulation of VSOACs

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Anion channels modulated by changes in cell volume are ubiquitously expressed and participate in cell volume homeostasis. Over the years, these channels have suffered from maladies of nomenclature, regulation and molecular identity. Since they have been studied in such a wide variety of vertebrate cell types by so many different investigators, they have acquired an almost equal variety of different names and acronyms. Thus, terms such as volume-regulated anion channel (VRAC), volume-sensitive Cl^- channel (VSCC) and volume-sensitive outwardly rectifying anion channel (VSOAC) have been used (often interchangeably) to describe outwardly rectifying anion channels activated by cell swelling. VSOAC should be the preferred acronym for the 'classic' type of volume-sensitive outwardly rectifying anion channel that resembles those initially described in lymphocytes (Cahalan & Lewis, 1988), since new evidence is emerging demonstrating the expression of a separate class of volume-sensitive *inwardly* rectifying anion channels in many cell types as well. Should we call these VSIACs (volume-sensitive inwardly rectifying anion channels) or simply AIRs (anionic inward rectifiers)?

There is a lack of consensus on the role of several key intracellular signalling pathways in the regulation of VSOACs in different cell types, and on the nature and fundamental properties of the volume sensor involved (Strange *et al.* 1996; Nilius *et al.* 1996; Okada, 1997). Some of this variability might well be explained by expression of molecularly distinct forms of VSOAC, regulatory or accessory proteins in different cell types. However, it seems prudent in future studies of VSOAC regulation to at least attempt to minimize some of the most obvious possible sources of variability. For example, do agents that modulate VSOACs act directly on the channel or a pathway or regulatory protein that regulates the channel, or are they simply modulating the degree of cell volume change in response to anisotonic solutions? Are some of the reported discrepancies in the regulation of VSOACs by various signalling pathways across different cell types due to contamination by overlapping Cl^- channel subtypes or cation channels? Finally, do agents modulate VSOACs under isotonic conditions or only after VSOACs are activated by cell swelling (to distinguish between signalling pathways similar to or distinct from those which normally link changes in cell volume to channel regulation)?

Not surprisingly, the identification of molecular candidates responsible for VSOACs has been replete with controversy. The list of possible molecular candidates first swelled, but then in recent years shrank (Clapham, 1998). New efforts to assess the relationship between both types of volume-sensitive anion channels (inwardly and outwardly rectifying channels of a known function, in search of molecular structure) and the ClC superfamily of voltage-dependent anion channels (channels of known molecular structure, many in search of function) are beginning to provide credible insights (Valverde, 1999). ClC-3, a ubiquitously expressed member of the ClC Cl^- channel family, is presently under consideration as a molecular candidate for VSOACs (Duan *et al.* 1997), and a specific N-terminal protein kinase C phosphorylation site has been proposed to act as the volume sensor (Duan *et al.* 1999). Certainly, the establishment of a minimal set of objective criteria (Okada *et al.* 1998) for the molecular identification of VSOACs and/or VSIACs will aid in this venture.

VSOACs and ClC-3 are both expressed in vascular endothelial and smooth muscle cells. It has been suggested that VSOACs may contribute to the development of myogenic tone (Nelson, 1998), but this hypothesis has been difficult to adequately test due to the lack of specific pharmacological inhibitors of VSOACs or ClC-3. In this issue of *The Journal of Physiology*, Ellershaw *et al.* provide evidence for novel regulation of VSOACs in smooth muscle cells of rabbit portal vein by nitric oxide (NO). NO and the NO donor SNAP inhibited VSOACs through a cGMP-independent pathway in approximately 50% of the cells examined, whereas NO and SNAP stimulated VSOACs in other cells through a cGMP-dependent mechanism. Although such variable effects might, at first sight, be attributed to complications related to technical limitations or problems with experimental design or techniques, dual opposing effects of NO on smooth muscle function have been observed previously. Furthermore, appropriate precautions appear to have been undertaken in the experiments by Ellershaw *et al.* (2000) to prevent untoward sources of variability. For example, NO, SNAP and 8Br-cGMP were shown not to alter the degree of cell swelling in response to a given hypotonic solution, thus eliminating the possibility that the effects on VSOACs observed might be indirect and merely due to drug-induced alterations in cell volume. This is particularly important in the case of cGMP, which in some types of cell may elicit changes in cell volume due to inhibition of Na^+–K^+–$2Cl^-$ cotransport (Clemo & Baumgarten, 1995). Likewise, possible contamination of macroscopic VSOAC currents by Ca^{2+}-activated Cl^- currents was minimized in a separate series of experiments in which intracellular Ca^{2+} was strongly buffered. Finally, pre-activation of VSOACs by cell swelling was shown to be required to demonstrate either the stimulatory or the inhibitory effect of NO on VSOACs, suggesting that NO signalling to VSOACs may be distinct from the normal volume sensor of the channel.

The exact physiological role of NO regulation of VSOACs in vascular smooth muscle remains to be determined. VSOACs may contribute importantly to resting Cl^- conductance in vascular smooth muscle and hence resting membrane potential. Thus the inhibition of VSOACs by NO is expected to cause membrane hyperpolarization and vasodilatation. The inhibition of VSOACs by NO demonstrated by Ellershaw *et al.* (2000) might well explain previous observations that disruption of the endothelium may greatly enhance Cl^--dependent noradrenaline-induced contractions (Lamb & Barna, 1998). VSOACs may, therefore, represent a novel target for NO in vascular smooth muscle cells and should be added to the growing list of cellular mechanisms believed to contribute to NO-induced vasodilatation. Although the physiological role of NO-induced stimulation of VSOACs in vascular smooth muscle is less certain, one can speculate that long-term effects might be related to regulation of cell proliferation (Voets *et al.* 1995) and apoptosis (Souktani *et al.* 2000). All these possibilities require additional study. It will be interesting to determine whether native VSOACs in other cell types exhibit a similar sensitivity to NO.

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