Science Signaling
AAAS

www.sciencesignaling.org/cgi/content/full/3/142/ra73/DC1

Supplementary Materials for

Nonsynaptic Communication Through ATP Release from Volume-Activated Anion Channels in Axons

R. Douglas Fields* and Yingchun Ni

*To whom correspondence should be addressed. E-mail: fieldsd@mail.nih.gov

Published 5 October 2010, *Sci. Signal.* **3**, ra73 (2010)
DOI: 10.1126/scisignal.2001128

The PDF file includes:

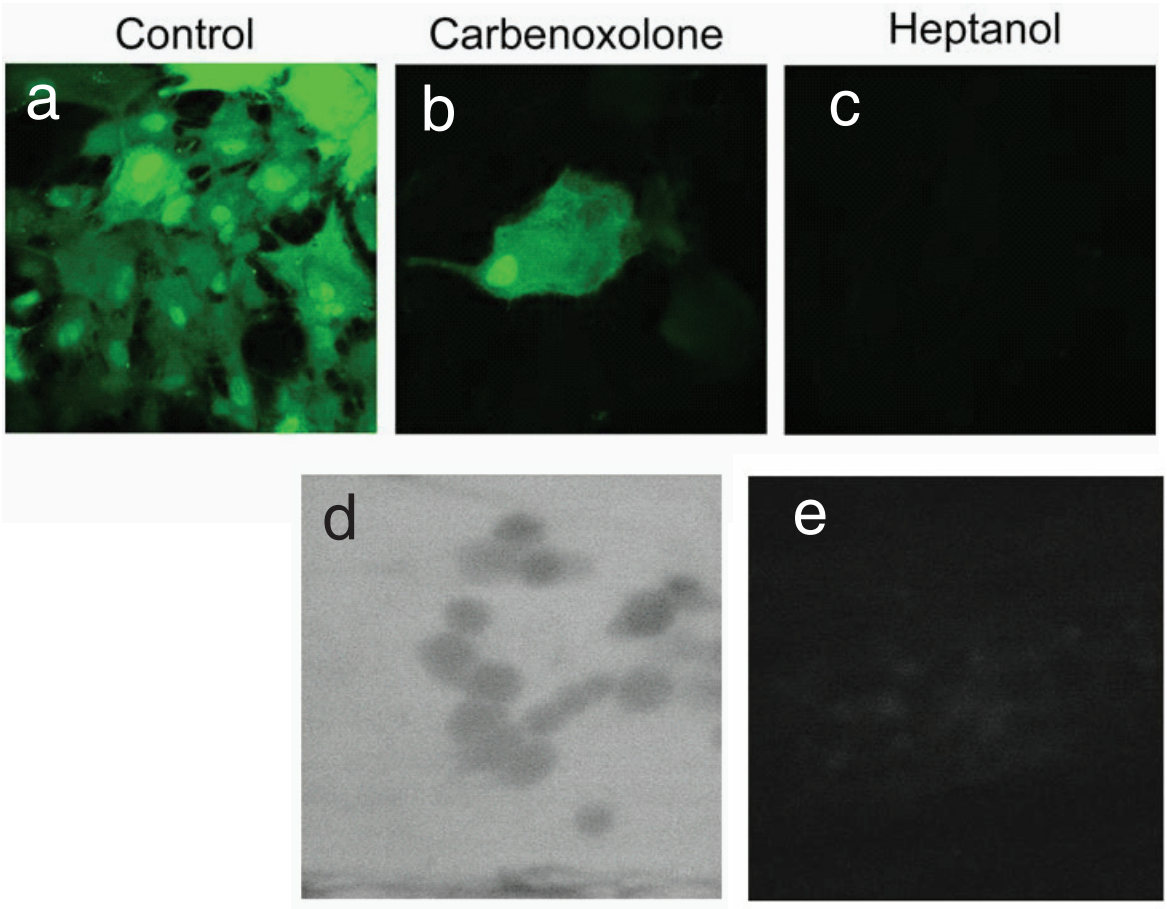

Fig. S1. Absence of functional gap junction hemichannels in DRG neurons and positive controls. Astrocytes incubated with 1 mM Lucifer yellow (LY) (Sigma) for ten min at 37°C readily take up the dye (a). Pre-incubation in either carbenoxolone (b) or heptanol (c) (100 μM, 20 min) inhibits loading of LY into the cells and the intercellular transfer of dye between cells. This positive control shows that the dye enters the cell through gap junction hemichannels and that the drugs are effective in blocking these channels. In contrast, there is no dye loading into DRG neurons, even when 10X higher concentrations of LY and incubation times twice as long as those used to load astrocytes are used. (d) twenty min after incubation in 10 mM LY while neurons are stimulated at 10 Hz. (c) 25 min after washing out LY there is no uptake of the dye into neurons.

Biophysically Identified Chloride Channels	Pharmacological Blockers
Maxi-anion channel	
swelling-induced ATP and glutamate release from non-excitable cells cell volume regulation gene identity unknown, VDAC (voltage-dependent anion channel) gene disputed as maxi-anion channel large-conductance (400 pS), 1.3 nm pore radius	arachidonic acid, DIDS, Gd^{3+}, NPPB, SITS (not sensitive to glibenclamide, phloretin)
VRAC (Volume-regulated anion channel)	
also known as VRC, VSOAC, VSOR cell volume and voltage-dependent ATP conductance cell volume regulation efflux of organic osmolytes, including glutamate, from non-excitable cells gene identity unclear; may not be a single entity intermediate conductance (90 pS at positive potentials), 0.63 nm pore radius	DCPIB, DIDS, glibenclamide, NPPB, phloretin, quinine, tamoxifen,
CFTR (cystic fibrosis transmembrane conductance regulator)	
cAMP regulated Cl^- channel implicated in ATP release regulates several conductances, including CaCC, VRAC, ORCC, and TRPV4 0.6-1 nm pore radius	glibenclamide (not sensitive to Gd^{3+})
ClC family	
9 genes with alternative splicing, forming homo and heterodimeric channels Found in intracellular organelles and plasma membrane ClC2 activated by cell swelling ClC3 relation to VRAC is disputed ClC 3/5 is a candidate for VAAC ClC3 not sensitive to DIDS and NPPB	Cd^{2+}, DCPIB, DIDS, niflumic acid, NPPB, Zn^{2+},
CACC (Calcium-activated chloride channel)	
molecular identity unclear; bestrophins (4 genes) and anoctamins (10 genes) implicated as candidate CACC modulated by CamKII	DIDS, flufenamic acid (FFA), NPPB, SITS, (not sensitive to phloretin)

Table S1. Guide to chloride channels with emphasis on those implicated in release of ATP and small amino acids during cell volume regulation. Other channels that have not been implicated in volume regulation include ligand-gated chloride channels (GABA, glycine receptors), acid-activated chloride channels (SLC26A7 gene candidate), cGMP-dependent CaCC, intracellular chloride channels (ClCs), ORCC (outwardly rectifying chloride channel), transporter-associated (ClCs), Calcium-activated chloride channel (ClCA family). Abbreviations: DCPIB 4-(2-butyl-6,7-dichlor-2-cyclopentyl-indan-1-on-5-yl); DIDS 4,4'-diisothiocyanostilbene-2,2'-disulphonic acid; FFA flufenamic acid; NPPB 5-nitro-2-(3-phenylpropylamino)benzoic acid; SITS 4'-isothiocyanostilbene-2,2'-disulphonic acid; ORCC outwardly rectifying chloride channel; VACC volume-activated chloride channel; VRAC volume-regulated anion channel (also known as porin); VRC volume-regulated channel; VSOAC volume-activated organic osmolyte-anion channel; VSOR volume-sensitive outwardly rectifying anion channel

Data compiled from information in:

1. S. P. H. Alexander, A. Mathie, J. A. Peters, Guide to receptors and channels, 4th edition. *British J. Pharm.* **158**, S1-S254 (2009).
2. C. Duran, C. H. Thompson, Q. Xiao, H.C. Hartzell, Chloride channels: often enigmatic, rarely predictable. *Ann. Rev. Physiol.* **72**, 95-121 (2010).
3. R. Z. Sabirov, Y. Okada, The maxi-anion channel: a classical channel playing novel roles through an unidentified molecular entity. *J. Physiol. Sci.* **59**, 3-21 (2009).