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6. AUTHOR(S)
Frederick Sachs

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
State University of New York at Buffalo
Sponsored Programs Administration
UB Commons, Suite 211
Amherst, NY 14228

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13. ABSTRACT

During tenure of the grant we have made tremendous strides in finding active ligands for the mechanosensitive channels. We developed a simple screen for the toxins based upon hypotonic swelling of GH3 clonal neurons which produced increases in internal Ca^{2+} levels that could be measured using Fura-2. Addition of active venoms would lead to a decrease in Ca^{2+} levels following swelling. Screening a variety of spider and scorpion venoms, we found that none of the scorpions tested ($\approx$ 12) but one of the $\approx$ 8 spiders tested was able to block volume activated Ca^{2+} uptake. The raw venom also blocked stretch activated ion channels in *Xenopus* oocytes, chick heart cells and GH3 cells, and whole cell mechanical currents in chick heart cells.

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Abstract:

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Publications arising from USARO support on this grant:

Chen, Y., S.M. Simasko, J. Niggel, W.J. Sigurdson, and F. Sachs (1996) Ca^{2+} uptake in GH3 cells during hypotonic swelling: the sensory role of stretch-activated ion channels. Am.J.Physiol. *270*:C1790-C1798.

Diamond, S.L., F. Sachs, and W.J. Sigurdson (1994) The mechanically induced calcium mobilization in cultured endothelial cells is dependent on actin and phopholipase. Arteriosclerosis and Thrombosis *14*:2000-2009.

Hu, H. and F. Sachs (1993) Effects of mechanical stimulation on embryonic chick heart cells. Upstate NY Cardiac Electrophysiology Society (Abstract)

Hu, H. and F. Sachs (1994a) Effects of mechanical stimulation on embryonic chick heart cells. Biophys.J. *66*:A170(Abstract)

Hu, H. and F. Sachs (1994b) Characterizing whole-cell mechanosensitive currents in chick heart. The Physiologist *37*:A9(Abstract)

Hu, H. and F. Sachs (1994c) Whole cell mechanosensitive current and its correlation with mechanosensitive channels in chick heart. Upstate NY Cardiac Electrophysiology Society (Abstract)

Hu, H. and F. Sachs (1995) Whole cell mechanosensitive currents in acutely isolated chick heart cells: correlation with mechanosensitive channels. Biophys.J. *68*:A393(Abstract)

Hu, H. and F. Sachs (1996a) Mechanically activated currents in chick heart cells. J.Membr.Biol. *154*: (in press)

Hu, H. and F. Sachs (1996b) Single-channel and whole-cell studies of mechanosensitive currents in chick heart. Biophysical J. *70*:A347(Abstract)

Izu, Y.C., S. Simasko, and F. Sachs (1993) Calcium pathways involved in the hypotonicity-induced calcium increase in GH3 cells. FASEB Journal *7(4)*:A891(Abstract)

Izu, Y.C. and F. Sachs (1994) Hypotonic cell swelling induced intracellular calcium increase and whole cell currents in GH3 cells: a response to mechanical stimulus and a mechanism for volume regulation. Upstate NY Cardiac Electrophysiology Society (Abstract)

Nazir, S.A., D.J. Dick, F. Sachs, and M.J. Lab (1995) Effects of *G. spatulata* venom, a novel stretch-activated channel blocker, in a model of stretch-induced ventricular fibrillation in the isolated heart. Circ. *292*:I-641, #3076(Abstract)

Niggel, J., H. Hu, W.J. Sigurdson, C. Bowman, and F. Sachs (1996) *Grammostola spatulata* venom blocks mechanical transduction in GH3 neurons, *Xenopus* oocytes and chick heart cells. Biophysical J. *70*:A347(Abstract)

Ruknudin, A., F. Sachs, and J.O. Bustamante (1993) Stretch-activated ion channels in tissue-cultured chick heart. Am.J.Physiol. *264*:H960-H972.

Ruknudin, A.M., H. Hu, and F. Sachs (1994) Extracellular ATP modulates stretch-activated channels in chick heart cells. The Physiologist *37*:A6(Abstract)

Sachs, F. (1993a) Ligands for stretch activated ion channels. 6th Int.Symp.Nondestructive Eval.Mat. 124(Abstract)

Sachs, F. (1993b) Stretch activated whole cell currents - whole cell correlates. Trans.Bioelec.Repair Growth Soc.(Abstract)

Sachs, F. (1994) Modeling mechanical-electrical transduction in the heart. In V.C. Mow, F. Guliak, R. Tran-Son-Tray, and R.M. Hochmuth (eds): Cell Mechanics and Cellular Engineering. New York: Springer Verlag, pp. 308-328.

Sachs, F. (1995a) A low drift micropipette holder. Eur.J.Physiol. *429*:434-435.(Abstract)

Sachs, F. (1995b) Mechanically sensitive ion channels: biological models for nanoscale stress sensors. Nondestr.Charac.Mater.6 621-628.

Sachs, F., F. Qin, and P. Palade (1995) Models of Ca^{2+} release adaptation. Science *267*:2010-2011.

Sachs, F. and H. Hu (1993) Stretch sensitive whole cell currents are alive and well. Ann.Biomed.Eng. *21*:39(Abstract)

Sigurdson, W.J., F. Sachs, and S.L. Diamond (1993) Mechanical perturbation of cultured human endothelial cells causes rapid increases of intracellular calcium. Am.J.Physiol. *264*:H1745-H1752.

Yang, X.C., and F. Sachs (1993) Mechanically sensitive, non-selective, cation channels. In D. Siemen and J. Hescheler (eds): Non-selective ion channels. Heidelberg: Springer-Verlag, pp. 79-92.

Zabel, M., B.S. Koller, F. Sachs, and M.R. Franz (1996) Stretch-induced changes in the isolated heart: importance of the timing of stretch and implications for stretch-activated ion channels. Cardiovas.Res. *32*:120-130.

Izu, Y.C. (1994) A study of the sensory mechanism of cell volume regulation. PhD. Thesis, Biophysical Sciences, SUNY, Buffalo, NY.

Inventions:

Sachs,F., Bowman, C.B., Y. Chen-Izu. High Affinity Ligand for Mechanosensitive Ion Channels. SUNY disclosure submitted. Patent application submitted.

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Y. Chen-Izu, 1994, SUNY Biophysics.

H. Hu, 1996, SUNY, Biophysics