Supplementary Material

Results

Biochemical properties— In the partial reduction / cyanylation method (Wu et al, 1996; Qi et al, 2001), the reduction of a single disulfide bond followed by cyanylation of the free thiols renders the peptide bond on the N-terminal side of cysteine residues involved susceptible to cleavage. Full reduction then results in the production of three fragments with a unique combination of masses for a given bond, and mass mapping indicates the cysteine linkage.

MALDI-TOF MS indicated that peak 1 contained the intact peptide (Average MW 4561.10 Da), peaks 2 and 3 predominantly contained singly reduced /alkylated isoforms (+52 Da, mass increase for a single reduction and cyanylation), peak 4 contained predominantly a doubly reduced isoform (+104 Da) and peak 5 contained the fully reduced peptide (+156 Da). The masses observed from cleavage and reduction of peak 4 correspond to those expected for reduction of Cys4, Cys6, Cys30 and Cys37, which suggests a double reduction at bonds CysI-CysV and CysII-CysIV, further supporting the data obtained from the singly reduced forms.

Materials and Methods

Xenopus oocytes preparation, cRNA injections and electrophysiological measurements— Oocytes were kept at 19°C in ND96 solution supplemented with penicillin (6 µg/ml) and streptomycin (5 µg/ml). Currents were recorded within 2-4 days of cRNA injections. In a 0.3 ml perfusion chamber, a single oocyte was gently impaled with two standard glass microelectrodes (1-2.5 Mohm) filled with a 3 mM KCl solution and maintained under voltage-clamp using a Dagan TEV 200 amplifier. Stimulation, data acquisition, and
analysis were performed using pClamp software (Axon Instruments). All experiments were performed at a 19-21°C room temperature in ND96 solution.

Drugs- Drugs were stored as stock solutions at −20°C. 1mM recombinant PcTx1 was stored in distilled water. Bovine serum albumin (BSA, Sigma-Aldrich, USA) at 0.1% was added to control and toxin-containing solutions in order to prevent peptide adsorption.