Mechanism of Arachidonic Acid Activation of C1C-2 Chloride Channel.

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Introduction/Background:
The C1C-2 C1- channel is widely distributed and is activated by voltage, pH, PKA and arachidonic acid (AA). Recently, in stretch activated K? channels, a charged region required for AA activation was identified. A similar charged region exists in the C-terminal domain of C1C-2 C1-. Changing the pH of one side of planar lipid bilayer creates an asymmetric charge between the two membrane leaflets. Thus by the changing pH of the medium bathing the extracellular (trans) side of the channel, the mechanism of AA activation will be determined.

Hypothesis:
AA activates the C1C-2 C1- channel by binding to a charged region of the channel protein.

Methods:
lipid bilayer reconstitution was used to study the CqC-2 C1- channel at pH trans 3.0 and 7.4. Two truncation mutants of the C1C-2 C1- channel were also studied: one with the charged region removed and one with it present. The effect of AA on open probability (Po) of the wild-type and mutant channels was measured with PKA activation as a control.

Results:
AA significantly activated wild-type C1C-2 C1 channel activity at both pH trans 3.0 ($P<0.01$) and 7.4 ($P<0.01$), as did PKA (control). AA did not activate the truncation mutant lacking the proposed binding site, but PKA activated the channel. In contrast AA produced significant activation ($P<0.01$) of the second truncation mutant.

Conclusion:
AA is an activator of the C1C-2 C1- channel. A specific charged region of the channel is necessary for this activation. Since channel activation occurs both at pHtrans 3.0 and 7.4, activation was not due to asymmetric binding of AA to the membrane, but rather to AA binding to the channel protein in the charged region.