Title: VOLATILE ANESTHETICS GATE A NOVEL CHLORIDE CONDUCTANCE IN CULTURED POST-NATAL RAT HIPPOCAMPAL NEURONS.

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Volatile anesthetics (VA) have been shown to decrease neuronal excitability by direct action on regenerative membrane activities, synaptic transmission, and resting membrane potential. Previous reports indicate that in both vertebrate and invertebrate central neurons, VA hyperpolarize the cell by activating a specific K^+ conductance. We report that in cultured rat hippocampal neurons, VA appears to gate a novel Cl^- conductance.

Experiments were conducted on enzymatically dissociated hippocampal neurons isolated from 1-2 day old post-natal rats, cultured for 3-4 days in vitro. Electrophysiological recordings were obtained with the standard whole cell patch-clamp technique. VA solutions (isoflurane or halothane) at various concentration diluted from a saturated solution at room temperature, was applied to the neurons by local microperfusion.

With symmetric Cl^- across the membrane, VA gates a non-desensitizing, outwardly rectifying, current in a dose-dependent manner. The outward rectification is more pronounced with decrease in internal $[Cl^-]$. The current is not an artifact of pressure or pH, it persists in the absence of external Ca^{++}, and is not blocked by picrotoxinin (1mM) or strychnine (10 uM). These properties distinguish I(Cl,anes) from the well known barbiturate induced Cl^- current (a GABAmimetic current blocked by picrotoxinin), the glycinergic current, or the phorbol ester sensitive voltage-gated Cl^- current previously shown to be present in these neurons. Although I(Cl,anes) does not require external Ca^{++}, we have not ruled out the possibility of activation of this current secondary to the internal release of Ca^{++}.

The strong outward rectification of this current and the normal Cl^- equilibrium potential near the cell's RMP provides a mechanism by which VA can selectively depress depolarized neurons with high activity level while not affecting quiescent neurons.

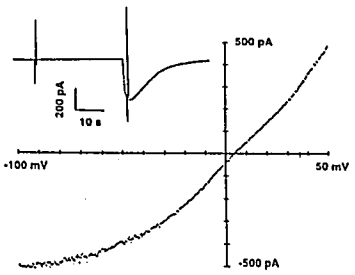

Figure 1: Ramp IV curve of isoflurane gated Cl^- current. V_{mem} was ramped (0.188 V/s) from -100 to +50 mV during peak response to a brief application of 1.7mM isoflurane. $[Cl^-]_o$ = 153 mM and $[Cl^-]_i$ = 145 mM. TTX and 4 mM $MgCl_2$ were present on the outside and CsCl inside to block voltage gated conductances. Inset: chart record tracing of ramp response before (left) and during (right) isoflurane application.

Title: Microdialysis measurements of arachidonic acid metabolites in rat CA1 hippocampus during transient cerebral ischemia.

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Arachidonic acid metabolites (Aam) are thought to aggravate ischemic brain damage by causing cerebral vasoconstriction with resulting hypoperfusion and by promoting tissue edema. However, the role of Aam remains unclear partly due to the obvious difficulty in correlating their postulated physiological effects in postischemic brain with concentration measurements obtained from 'downstream' sites (i.e. blood, csf). Direct brain tissue sampling is an alternative but may itself provoke Aam production and introduce artifacts. Microdialysis, on the other hand, is in principle the only technique which can provide continuous substance concentration measurements from any brain region. In the following, we report our preliminary experience with brain Aam measurements (thromboxane) in rat hippocampus during cerebral ischemia using microdialysis.

Male Sprague-Dawley rats (300-400 grams) were prepared for the four vessel occlusion model of cerebral ischemia. Under halothane anesthesia, the vertebral arteries were cauterized on day 1. On day 2, rats were placed in a stereotaxic apparatus and a microdialysis probe was placed in the left CA1 hippocampus. The probe was perfused with mock csf at a flow rate of 2.5 µl/min. and dialysates were collected at 20 min. intervals for thromboxane (TxB_2) analysis by RIA. Complete cerebral ischemia was induced by snaring the carotid arteries for 20 min. and maintaining mean arterial pressure at 80 mmHg.

The figure demonstrates dialysate TxB_2 concentrations (pg/ml) from hippocampus before, during and after 20 min. of complete cerebral ischemia. Note that the large and significant TxB_2 increase first occurs upon reperfusion and gradually returns to baseline after two hrs. Plasma TxB_2 concentrations in experimental animals were not significantly different from those of sham operated controls at any time.

Our study demonstrates for the first time direct measurements of Aam in the brain interstitial space. Membrane bound arachidonic acid (Aa) is liberated shortly after the onset of ischemia, and, upon reperfusion with the availability of O_2, cyclo- and lipoxygenase enzymes can act on Aa to produce a burst production of Aam. This appears to be confirmed by our results. The large increase of the potent vasoconstrictor TxB_2 raises some important questions in relation to the postischemic hypoperfusion phenomena. These issues and the possible relationship between Aam and the release of excitatory neurotransmitters in ischemic brain are the subject of ongoing investigations.