Modulation of Afterhyperpolarization by Recurrent Inhibition

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Afterhyperpolarization (AHP) is a major determinant of motoneuron (MN) firing rate. However, it is not the sole factor since it is shaped by excitatory and inhibitory synaptic conductances during different motor tasks. One likely candidate is recurrent inhibition which shared similar timing with the known undershoot of MN action potentials following antidromic stimulation of peripheral nerve. That observation prompted us to directly examine the extent by which recurrent inhibition contributes to MN hyperpolarization. Data were obtained by in vivo intracellular recordings from identified lumbar MNs. Recordings were obtained from 63 MNs in 16 adult rats acutely deafferented and Isoflurane anesthetized. In each MN, action potentials were evoked either by suprathreshold current injection or by antidromic electrical stimulation of the whole peripheral nerve activating the autogenic recurrent inhibition circuit. Changes in the degree of peak hyperpolarization, rate to negative peak, and rate of decay were investigated. Recurrent inhibition added an average increase of 646µV to the peak hyperpolarization ranging from 18µV to 3.46mV. That corresponds to an average increase of 46.5% and up to 1.64 times of original AHP amplitudes. The mean rate of rise increased by 1.53 times ($P<0.0001$) and the mean rate of decay increased by 1.4 times ($P=0.0018$). Collectively, recurrent inhibition addition to AHP resulted in significantly larger and faster hyperpolarization following action potentials. That modification is likely to influence firing (rate, the ability to fire for longer durations) and provides direct evidence that further validates the method used to measure recurrent inhibition in humans which pre-requires that recurrent inhibition magnitude is not negligible when compared to that of AHP
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Pharmacology and Toxicology

ETI-385 as a Novel Anti-emetic Against Drug Induced Emesis

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Drugs currently used to prevent emesis (nausea and vomiting) target only one or a few of the pathways used by emetic stimuli to trigger the reflex. Thus, an anti-emetic drug will only be effective against some stimuli. Prior work determined that 8-OH-DPAT (DPAT, a 5-HT1A agonist) was a universally effective anti-emetic. Although DPAT prevented emesis, it also elicited an extreme anxiety response making it unsuitable for therapeutic use. Presently, there exists no universal anti-emetic drug. We tested a proprietary drug developed from DPAT, ETI-385, which successfully prevented emesis in musk shrews against chemotherapy, drug and motion stimuli. For FDA purposes, we are required to test ETI-385 in another species before taking the drug into clinical trials. Work at Epiomed Therapeutics used ETI-385 to successfully prevent emesis in cats using motion stimuli. Currently, ETI-385 is being tested in cats against a drug stimulus, Xylazine, which is a common veterinary sedative. We determined a dose response curve for ETI-385 against Xylazine over the range of 0.0225mg/kg to 0.36mg/kg. The animals received an ETI-385 pretreatment injected subcutaneously (SC) followed by an injection of Xylazine (also SC). During observation the animals were scored for symptoms of both emesis and anxiety. The dose

0.0225mg/kg was unsuccessful in preventing emesis and produced a higher symptom score than Xylazine alone, suggesting nausea. The dose 0.045mg/kg was 66.7% effective against vomiting but had an increase in symptom score while 0.09mg/kg was 83.4% effective against vomiting with a marked decrease in symptom score. At the 0.36 mg/kg dose we achieved 100% efficacy and a complete eradication of emetic symptoms. Unlike DPAT, only the highest dose produced any defensive behavior.

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Hs3A/hs1,2 or Hs3B/hs4 is Sufficient to Mediate TCDD-Induced Inhibition Of the 3'IgH In A Transgenic B-Cell Line

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Immunoglobulin (Ig) gene expression is inhibited by AhR ligands including TCDD in both in vivo and in vitro animal models and human cellular models. In mouse models, Ig inhibition correlates with AhR expression and function. Ig heavy chain (Igh) gene expression involves a complex interaction between several regulatory elements including the 3'Igh regulatory region (3'IghRR), which is typically associated with four enhancers (hs3A; hs1,2; hs3B; hs4). We have demonstrated in a mouse B-cell line CH12.LX that TCDD inhibits LPS activation of luciferase reporters regulated by the 3'IghRR or the hs1,2 enhancer alone. Surprisingly, a luciferase reporter regulated by the hs4 enhancer was synergistically activated by LPS and TCDD. The objective of this study was to determine in the context of chromatin if the inhibitory effect of TCDD is mediated through the hs1,2 enhancer. CH12.LX cells were stably transfected with constructs containing an LPS-inducible γ2b reporter regulated by the 3'IghRR with LoxP sites flanking either the hs3B/hs4 or the hs3A/hs1,2 enhancer pairs. Transfection with CRE-recombinase induced LoxP recombination generating cell lines either expressing γ2b regulated by hs3A/hs1,2 or by hs3B/hs4. TCDD inhibited LPS-induced activation of both the parental 3'IghRR and the deletional derivatives, hs3A/hs1,2 and hs3B/hs4, suggesting that either enhancer pair can mediate the inhibitory effect of TCDD on the 3'IghRR but together do not produce a synergistic or additive effect. These results are in contrast to the effect of TCDD on the hs4 luciferase reporter which may be due to the addition of hs3B in the stable reporter. In conclusion, it appears that either 3'IghRR enhancer pair can be targeted by TCDD and is sufficient to mediate the inhibitory effect on 3'IghRR activity. Altered 3'IghRR activity likely contributes to the TCDD-induced inhibition of the antibody forming cell response due to the well-established association between the 3'IghRR, Igh expression, and antibody levels. (Supported by NIEHS R01ES014676)

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Localization of Renin Angiotensin Peptidases In Mouse Kidney Using Dual Immunofluorescence and Microscopy

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The kidney renin angiotensin system (RAS) plays a pivotal role in the regulation of blood pressure. There is emerging evidence that the two main Ang peptide mediators, angiotensin (Ang) II and Ang-(1-7), have counter-regulatory roles. While Ang II functions as a potent vasoconstrictor, the vasodilator Ang-(1-7) has been found to protect