(LTCCs) are important in activity-dependent neurite outgrowth, which comprises neurite initiation and elongation. We used cerebellar granule neurons (CGNs) to differentiate between LTCC effects on neurite initiation vs elongation. We also tested cerebellar function in mice lacking specific LTCCs with behavioral assays. METHODS/STUDY POPULATION: CGNs were cultured from 129SvEv mouse pups at P4-P6. Potassum chloride (50mM) was used to stimulate neuronal cultures for 24 hours. Isradipine (20nM) was added to culture medium to inhibit all LTCCs for 1 hour. For Cav1.2 deletion, we crossed Cav1.2 conditional knockout mice (Cav1.2-cKO) to Syn-Cre mice (for deletion in most neurons) or Atoh1-Cre mice (for deletion in CGNs). The Cav1.2-cKO line was maintained on a 129SvEv background. For constitutive Cav1.3 deletion, mice were maintained on a C57BL/6NTac. Behavioral tasks included open field, rotarod, and Erasmus Ladder. Data were analyzed with sexes combined and separated to assess for sex as a biological variable. Studies were analyzed by one-way ANOVA, two-way ANOVA, or generalized linear mixed model, where appropriate. RESULTS/ANTICIPATED RESULTS: CGNs exhibited an increase in neurite initiation but not elongation when stimulated with potassium chloride, consistent with previous reports of activity-dependent neurite outgrowth in this cell type. LTCC inhibition with isradipine blunted KCl-induced neurite initiation. We observed no change in the length of either primary or secondary neurites with isradipine treatment with or without KCl stimulation. In our behavioral experiments, we observed no deficits in open field, rotarod, or Erasmus Ladder when Cav1.2 was deleted in most neurons (driven by Syn-Cre expression) or in cerebellar granule neurons (driven by Atoh1-Cre expression). In contrast, loss of Cav1.3 was associated with impaired motor learning in the rotarod task without evidence of ataxia on Erasmus Ladder. DISCUSSION/SIGNIFICANCE OF FINDINGS: We show a specific role for LTCCs in activity-dependent CGN neurite initiation. While loss of Cav1.2 does not affect motor learning, loss of Cav1.3 does impair motor learning. Our results help expand our understanding of LTCC function in cerebellar neurodevelopment and function.

21813

Changes in Electrophysiologic Activity in the Rat Visual Cortex following Traumatic Brain Injury (TBI)

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ABSTRACT IMPACT: This research aims to identify changes in visual network function after TBI as a way to define potential therapeutic targets for neuromodulation or neural tissue substrates. OBJECTIVES/GOALS: The objectives of this study are to compare neural activity in the visual cortex following TBI with cortical activity in the uninjured brain. This study aims to characterize functional changes in single neuron activity, spike-field relationships and oscillatory activity. METHODS/STUDY POPULATION: The effects of TBI will be studied by comparing electrophysiologic recordings from Long-Evans rats with a fluid percussion injury (FPI) to rats with a sham injury. Four days after the injury or sham procedure, a laminar probe with multiple electrode contacts will be chronically implanted in the ipsilesional primary visual cortex (V1). Afterwards, rats will be anesthetized weekly for 3 weeks (up to 4 weeks post-injury) to assess visual processing in response to drifting grating visual stimulation. To assess behavioral correlates, neural activity will also be recorded while rats perform a visual discrimination task in an operant, touchscreen chamber twice weekly. Recordings will be analyzed for visually evoked units, unit entrainment to local field potentials (LFPs) and evoked oscillatory activity. RESULTS/ANTICIPATED RESULTS: Consistent with other studies, our preliminary evidence from V1 recordings in naive rats has shown that individual neurons are responsive to visual stimuli, visual stimuli are associated with evoked oscillations and unit activity is correlated with LFPs. While activity of individual V1 neurons in injured animals is expected to recover to resemble activity in uninjured animals over time, patterns of functional organization in the two groups are expected to diverge over time. We anticipate that TBI-associated axonal damage, neuronal loss and changes in synaptic weights will lead to disruptions in the timing of neural activity in V1. These perturbations of neural communication within the visual system are expected to be associated with behavioral deficits in the awake, visual discrimination task. DISCUSSION/SIGNIFICANCE OF FINDINGS: This study helps define how cortical network disruption after TBI. These changes are potential targets for novel TBI therapeutics, including neuromodulation and neural tissue transplantation. Thus, this work lays the groundwork for future studies aimed at mitigating the effects of TBI with rationally designed experimental therapeutics.

24088

Investigation of the Apelingeric System on Oxidative Imbalance within Cardiorenal Syndrome Type 4

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ABSTRACT IMPACT: Approximately 15% of US adults have chronic kidney disease with over 700,000 of those in the end stages where treatment options are severely limited to dialysis or kidney transplant; the research presented here will help identify novel strategies that address oxidative imbalance and preserve renal function. OBJECTIVES/GOALS: Chronic Kidney Disease patients often develop secondary cardiovascular disease - Cardiorenal Syndrome Type 4. RNA sequencing data show increased apelin receptor expression in 5/6 nephrectomy rats. The Apelinergic (APJ) system is deemed beneficial in normal physiological systems. Here we explore links between stress and the APJ system in CRS4. METHODS/STUDY POPULATION: In preliminary studies performed in NRK cells, inflammatory cytokines, IL-1β and IL-6, caused increases in apelin receptor transcripts and decreased apelin transcripts, respectfully. The literature describes inflammatory processes that contribute to degradation of many organs (kidneys, heart, and liver) suggesting an oxidative imbalance. To investigate this imbalance within CRS4, three rat cell types" H9c2 cardiomyocytes, HII4E hepatocytes, and NRK renal epithelial cells" will be used to assess the role of exogenous apelin on pro- and anti-oxidant levels. Cells will be pretreated with apelin or vitamin E 48 hours prior to the addition of toxins or cytokines (uremic: uric acid and d-galactose or hydrogen peroxide; cytokines: IL-1β and IL-6), to assess pro- and anti-oxidant protein levels via Western Blot. RESULTS/ANTICIPATED RESULTS: We anticipate with toxin or cytokine addition (either uremic, hydrogen peroxide, IL-1β or IL-6) in all cell types, an increase in protein levels for GPX" a known measure of oxidative stress" should be greater than the increases in antioxidants" SOD1 and Catalase. After pre-treatment of vitamin E, GPX protein levels should decrease compared to toxin/cytokine control, while SOD1 and catalase protein levels increase; this coincides with vitamin E inducing antioxidant activity in animals and humans. The anticipated results for this study after exogenous apelin addition should reveal in all three cells types reduced levels of GPX and increased levels of SOD1 and

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Catalase comparable to the vitamin E treatment. DISCUSSION/SIGNIFICANCE OF FINDINGS: If addition of apelin causes an antioxidant response in all three cells types, this can build on evaluation of the APJ system as a therapeutic option for those with CKD and CRS4 to minimize both inflammatory and oxidative stress. With the data gathered here, we expect to recreate the results in a CKD rat model that highlights these same manifestations.

25380

Cholecystokinin-B Receptor Mediates Growth of Hepatocellular Carcinoma*

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ABSTRACT IMPACT: Cholecystokinin-B Receptor Mediates Growth of Hepatocellular Carcinoma with the use proglumide. Proglumide is a non-selective antagonistic drug therefore, strategies that block signaling at the CCK-BR may provide to be a novel therapeutic option for Hepatocellular Carcinoma treatment OBJECTIVES/GOALS: Cholecystokinin (CCK)and gastrin mediate the growth of Hepatocellular Carcinoma (HCC) through CCK-R and interruption of this signaling pathway could decrease HCC. CCK-Receptors are overexpressed in HCC and proliferation may be mediated through CCK-B. Blockade of the CCK-BR with proglumide decreased both growth in vitro and tumor growth in vivo. METHODS/STUDY POPULATION: RNA was extracted from murine Hepa1-6, RIL-175 and human HepG2 cells and was evaluated by qRT-PCR for expression of CCK-AR, CCK-BR and gastrin. CCK-R protein expression was analyzed by flow cytometry. HCC cells were treated in vitro with CCK peptide, the CCK-AR antagonist or the CCK-BR antagonist. Proliferation of selective CCK-R KO cells was compared to that of wild-type cells. To determine the effect of a CCK-R antagonist on tumor growth in vivo two cohorts of mice bearing subcutaneous Hepa1-6 or RIL-175 HCC tumors were treated with an oral bioavailable CCK-R antagonist proglumide or untreated water for 3-4 weeks. The mice bearing Hepa1-6 tumors were placed on a high-fat diet to raise blood CCK levels. Mice bearing RIL-175 tumors were fed standard chow to determine if proglumide could block autocrine growth by gastrin. RESULTS/ANTICIPATED RESULTS: The mRNA expression of CCK-AR, CCK-BR and gastrin were increased 80-90-fold in all HCC cell lines compared to that of normal liver. CCK-BRs were detected on >85% of the cells by flow cytometry. CCK peptide (1nM) stimulated HCC growth in vitro in both wild-type cells and in CCK-AR KO cells but not in CCK-BR KO cells. CCK-BR antagonist blocked CCK-stimulated growth in vitro but the CCK-AR antagonist did not, suggesting that the CCK-BR was responsible for mediating proliferation. In vivo tumor growth was significantly reduced with proglumide treatment by 70% ($p<0.05$) in Hepa1-6 and by 73% ($p<0.001$) in RIL-75 tumors, respectively. DISCUSSION/SIGNIFICANCE OF FINDINGS: CCK-Rs are overexpressed in HCC and proliferation appears to be mediated through the CCK-BR. Downregulation with CRISPR Cas9 or blockade of the CCK-BR with an antagonist decreases growth in vitro and proglumide therapy decreases tumor growth in vivo. Strategies that block signaling at the CCK-BR maybe a novel therapeutic option for HCC treatment.

27558

Obeticholic acid (OCALIVA ®) protects against 2,8-dihydroxyadenine nephropathy in mice

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ABSTRACT IMPACT: This work may lead to new treatments for crystalline nephropathies. OBJECTIVES/GOALS: This study investigated obeticholic acid (OCALIVA ®) as a potential treatment for 2,8-dihydroxyadenine (2,8-DHA) nephropathy using a mouse model. The treatment was investigated in both sexes at two timepoints. METHODS/STUDY POPULATION: Male and female C57BL/6J mice (12 weeks of age) were fed chow (Research Diets D19120401i) or chow admixed with adenine (0.2% w/w) ad lib for either 3.5 or 7 weeks. Mice were treated with either vehicle (corn oil) or obeticholic acid (10 mg/kg BW) by gavage 5 days per week. Each of the 16 combinations of sex/diet/timepoint/treatment groups had an $n = 6$ (96 mice in total). Food and body weights were measured twice per week, and 24-hour urines were collected prior to euthanasia. Serum and organs were collected and processed for biochemical and histopathological analyses. RESULTS/ANTICIPATED RESULTS: At both the 3.5-week and 7-week timepoints, dietary adenine robustly increased BUN and serum creatinine compared to control diet in vehicle-treated male and female mice ($P < .01$, all comparisons). At the 3.5-week timepoint, obeticholic acid reduced BUN in male ($P < .05$) but not female adenine mice. Obeticholic acid did not affect serum creatinine at this timepoint. At the 7-week timepoint, obeticholic acid reduced BUN in female ($P < .05$) but not male adenine mice. At the 7-week timepoint, obeticholic acid reduced serum creatinine in both male ($P < .05$) and female ($P < .01$) mice. Biochemical and histopathological analyses are ongoing, and we anticipate that the results will agree with the serum chemistries. DISCUSSION/SIGNIFICANCE OF FINDINGS: Obeticholic acid is FDA-approved for primary biliary cholangitis, and it is in clinical trials for several other hepatobiliary diseases. Although currently untested in humans, it is nephroprotective in many preclinical models of kidney disease. This study is the first to investigate obeticholic acid in a model of crystalline nephropathy.

32219

Differences in cell death in methionine versus cysteine depletion

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ABSTRACT IMPACT: Reducing methionine levels has repeatedly been shown to reduce cancer growth in vivo, while at the same time increasing lifespan in healthy animals. However, the mechanisms behind the beneficial effects of methionine restriction are currently unknown. OBJECTIVES/GOALS: We hypothesized that comparing the response of a cancer cell line to depletion of the amino acids methionine and cysteine would give us insight into the critical role of these two closely related amino acids in cancer, and help advance methionine restriction on the translational science spectrum. METHODS/STUDY POPULATION: We used the human

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