
Southeastern Pharmacology Society 27th Annual Meeting Summary and Abstracts

The Southeastern Pharmacology Society met at the University of Mississippi for its 27th Annual Meeting on November 5-7, 2006. The meeting was a great success with a total of 53 enthusiastic attendees. The feedback at the meeting was extremely favorable with attendees indicating that they enjoyed the diverse areas of research presented.

Travel Awards were presented to individuals representing eight institutions: Mercer University, University of Mississippi Medical Center, Meharry Medical College, Mississippi State University, Medical College of Georgia, Harrison School of Pharmacy at Auburn University, University of Tennessee Memphis, and Pennington Biomedical Research Center.



Congratulations to the following award winners:

**First place winner for platform presentation:
Dr. Abir El-Alfy, *University of Mississippi***

**Second place winner for platform presentation:
John Bauer, *Mercer University***

**First place winner for poster presentation:
Jennifer King, *Meharry Medical School* and Ajay Sood, *Medical College of Georgia***

Special Thanks to the following "Volunteer" Judges who diligently visited all poster presentations and listened closely to the platform presentations:

Jerry Buccafusco, *Medical College of Georgia*
Clivel Charlton, *Meharry Medical College*
John Kermode, *University of Mississippi Medical Center*
Greg Ordway, *East Tennessee State University*
Zia Shariat-Mader, *University of Mississippi*



ABSTRACTS FROM THE SOUTHEASTERN PHARMACOLOGY SOCIETY 27TH ANNUAL MEETING:

NITRIC OXIDE-DONATING *m*-TERPHENYL AMINES; EN ROUTE TO A SAFER NSAID

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Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs despite their gastrointestinal and cardiovascular side effects. A series of *m*-terphenyl amines was synthesized and evaluated as a novel class of cyclooxygenase (COX) inhibitors. Structure-activity relationships (SAR) were investigated by functional group modification at the para-position of the C-1' and C-2' phenyl substituents on the central aromatic ring. Anilines 6a,b,d,h demonstrated nonselective inhibition of COX-1 and -2 in human whole blood. Compounds 6c,e demonstrated preferential inhibition of the COX-2 isozyme at 10 μ M. Molecules 6f,i,j were found to only inhibit COX-1; the disubstituted ethoxy derivative (6g) was inactive as a COX inhibitor (\leq 100 μ M). Molecular docking studies indicate that the COX-1 binding site amino acid Ile⁵²³ anchors the *m*-terphenyl system statically within the enzyme's active site, while the slightly smaller amino acid Val⁵²³ in COX-2 allows the ligand to "roll", fashioning several acceptable conformers. Therefore, studies are currently underway to investigate these tunable inhibitors by taking advantage of their flexible fit in the COX-2 active site over their more rigid binding in the COX-1 active site. Ultimately, this should afford more potent inhibitors with a spectrum of COX isozyme selectivity.

While selective COX-2 inhibitors have seemingly lost their allure, attention is refocusing on the use of hybrid NSAID nitric oxide (NO) donors for the treatment of inflammatory disorders. Compound 6a was subsequently converted into a NO-donating prodrug (NO-6a) utilizing an N-acetyl-D-penicillamine tether. NO-6a demonstrated a dose dependent inhibition (ED₅₀ ~75 mg/kg i.p.) of carrageenan induced hind paw edema in rats, which was approximately twice the potency compared to 6a. We are currently evaluating the gastrointestinal and cardiovascular safety of this novel NO-donating NSAID *in vivo*.

ACCESSION AND FERMENTATION OF ALASKAN FUNGAL ORGANISMS FOR THEIR METABOLITES

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During the course of evaluating microorganisms for biologically active natural products, we accessed several fungi from Alaska. Upon subsequent fermentation, pseurotin A (1) and LL-D253 α (2) were isolated from isolates JBAK-1 and JBAK-6, respectively. Structures were established from the interpretation of spectroscopic data. X-ray data for 2 revealed an enantiomeric mixture, 30(-):70(+), whereupon crystal packing is dictated by significant intra and intermolecular hydrogen bonding. Furthermore, 2 demonstrated antibacterial activity against methicillin-susceptible and -resistant *Staphylococcus aureus* strains.

MPTP AFFECTS ENDOGENOUS COENZYME Q CONTENT

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Objective: To study the effect of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) on the antioxidant/mitochondrial energy enhancer Coenzyme Q10 content in mice brain (*in vivo*) and in dopaminergic neuronal cells (*in vitro*).

Background: Parkinson's disease (PD) is a neurodegenerative disease and its prevalence increases with age. Oxidative stress in the substantia nigral region leads to the pathogenesis and progression of PD. Coenzyme Q10 is an endogenous lipophilic antioxidant biosynthesized in cells and plays an indispensable role in ATP synthesis. Coenzyme Q10 is currently used therapeutically to alleviate various neurological disorders. The effect of MPTP on coenzyme Q content has not been clearly established.

Material and Methods: SH-SY5Y (human) and NG108-15 (rodent) cells were cultured and used for the current study. Cell Proliferation assay (MTT) was carried out after the incubation with MPP⁺. Coenzyme Q content was assessed using HPLC-UV. For *in vivo* experiment, mice were administered with MPTP (30mg/kg, i.p., twice 16 hrs apart) and after one week, the animals were sacrificed for the measurement of coenzyme Q content in different brain region.

Results: MPP⁺ significantly increases the coenzyme Q-10 content dose dependently in both the cells. Coenzyme Q-9 and Q-10 was found in substantia nigra, nucleus caudate putamen, cerebrum, cerebellum and pons. Administration of MPTP to mice depletes dopamine in nucleus caudate putamen and alters coenzyme Q levels in the nigrostriatal tract but not in the other regions of the brain.

Conclusion: This study shows that dopaminergic neurotoxin MPTP alters the coenzyme Q-10 content and induces cell death. Results of this study suggest that neurotoxic insult by MPTP causes oxidative stress which results in the alteration of coenzyme Q10 content.

Acknowledgement: This study was supported by Department of Pharmacal Sciences Auburn University, Auburn, AL.



STRUCTURAL ACTIVITY RELATIONSHIP STUDIES OF ZEBRA MUSSEL ANTIFOULING AND ANTIMICROBIAL AGENTS FROM VERONGID SPONGES

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Several dibromotyramine derivatives including moloka'iamine were selected as potential zebra mussel (*Dreissena polymorpha*) antifoulants due to the noteworthy absence of fouling observed on sponges of the order Verongida. Sponges of the order Verongida consistently produce these types of bromotyrosine-derived secondary metabolites. Previously reported antifouling data for the barnacle *Balanus amphitrite* ($EC_{50} = 12.2 \mu M$) supports the results reported here that the compound moloka'iamine may be a potential zebra mussel antifoulant compound ($EC_{50} = 10.4 \mu M$). The absence of phytotoxic activity of the compound moloka'iamine toward *Lemna paucicostata* and most importantly, the compound's significant selectivity against macrofouling organisms such as zebra mussels suggests the potential utility of this compound as a naturally derived antifoulant lead.

EFFECT OF ENDOGENOUS AND EXOGENOUS TOXIN (COADMINISTRATION) IN MICE: BEHAVIORAL AND BIOCHEMICAL ANALYSIS

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Objective: To establish the chronic effect of endogenous (salsolinol) and exogenous (diquat) toxin on the behavior and monoaminergic neurotransmitters in mice.

Background: Chronic exposure to pesticides/herbicides results in accumulation of various toxic metabolic by-products, which has the relevant ability to cause symptoms of Parkinson's disease (PD). Environmental neurotoxins such as paraquat, rotenone and 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) have been implicated the etiopathology and progression of PD. Diquat (herbicide) is currently being widely used in USA and other parts of the world and structurally resembles MPTP/paraquat. Dopamine-derived endogenous neurotoxin salsolinol is also involved in the pathogenesis of PD. However, the behavioral and biochemical mechanisms underlying the chronic exposure to these endogenous & exogenous toxins have not been established.

Design/Method: C57/BL6 mice were divided into 4 groups and intraperitoneally administered with vehicle or toxins twice a week for six weeks. The groups are: [i] Control (sterile water), [ii] Diquat (10mg/kg), [iii] Diquat + Salsolinol (10+5mg/kg), [iv] Diquat + Salsolinol (10+10mg/kg). Behavioral parameters such as, akinesia, catalepsy, swim test, tremor, straub tail and rotorod were observed after the last dose of regimen. Striatum was dissected and analyzed for the monoaminergic neurotransmitters employing HPLC-ECD. *In vitro* effect of diquat on complex-I activity, monoamine oxidase and Reactive Oxygen Species (ROS) were evaluated. Statistical data analysis was performed using Sigma stat.2.03 software.

Results: Diquat + Salsolinol (high dose) significantly reduced the locomotor activity without altering the monoaminergic neurotransmitters (dopamine, serotonin and norepinephrine) in the striatum. Diquat (alone) did not affect complex-I activity in brain mitochondria. Diquat dose-dependently increased the generation of ROS.

Conclusions: Chronic exposure to Diquat + Salsolinol induced significant behavioral deficits. However, unlike other neurotoxins (rotenone, 6-hydroxydopamine or MPTP), diquat and/or Diquat + Salsolinol did not induce severe dopamine depletion in the striatum.

Acknowledgement: This study was supported by Department of Pharmacal Sciences, Auburn University, Auburn, AL.

BOTH THE PREVALENCE AND THE PATTERN OF LINKAGE DISEQUILIBRIUM BETWEEN THE POLYMORPHIC VARIANTS OF PLATELET GLYCOPROTEIN IB DIFFER GREATLY AMONG RACIAL GROUPS

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Interaction of von Willebrand factor (VWF) with circulating blood platelets is the initial trigger for thrombosis in a region of arterial stenosis. The primary binding site for VWF on the platelet is located on the α -chain of platelet glycoprotein Ib (Gplb α). Several polymorphisms have been reported in the gene for this glycoprotein. Two polymorphisms, in particular, appear to alter the risk of cardiovascular disease. One is a single nucleotide C/T dimorphism at nucleotide 482 in the Gplb α gene; it results in a change in the codon for amino acid residue 161 from threonine to methionine. This residue is located within the VWF binding domain on Gplb α . The other polymorphism involves a variable number of tandem repeats (VNTR) of a 39-nucleotide sequence encoding 13 amino acid residues in the extracellular portion of Gplb α ; this sequence may occur once (VNTR-D allele), twice (VNTR-C), three times (VNTR-B) or four times (VNTR-A). We have developed a new method based on a real-time polymerase chain reaction (PCR) with allele-specific primers to assay the ⁴⁸²C/T dimorphism. This method avoids the confounding effect of an adjacent ⁴⁸³G/A dimorphism that is thought to be silent. It has been validated by comparison with the standard procedure based on restriction digestion. We have genotyped individuals from several racial groups for these two dimorphisms by real-time PCR and for the VNTR polymorphism by the standard procedure of PCR and gel electrophoresis. Our findings indicate that the rarer ⁴⁸²T allele (usually encoding ¹⁶¹Met) is much more prevalent in African Americans than in Caucasians or Asians. The VNTR-B allele is also much more prevalent in African Americans. However, the pattern of linkage disequilibrium between these two polymorphisms is much weaker in both African American and Asian populations than in Caucasians. As the ¹⁶¹Met and VNTR-B variants of Gplb α seem to be associated with a higher risk of



cardiovascular disease, their increased prevalence in African Americans might contribute to the greater frequency of cardiovascular disease in this population. [Supported by the National Science Foundation and the Mississippi Functional Genomics Network.]

COMPARATIVE STUDY OF THE PROTECTIVE EFFECTS OF CALPEPTIN, N-ACETYL-L-CYSTEINE, AND TAURINE AGAINST ACRYLAMIDE-INDUCED NEUROTOXICITY IN RATS: POSSIBLE ROLES OF APOPTOSIS AND OXIDATIVE STRESS

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The work announced in Sweden, April 2002, that reported the presence of unacceptable levels of acrylamide in fried food raised the alarm of the scientific community. Such concern is due to the well established toxicities of acrylamide as a neurotoxin, carcinogen, and mutagen. The objective of this study was to determine if calpeptin, an established apoptosis inhibitor, N-acetyl-L-cysteine (NAC), a potent antioxidant, and taurine that acts both as an apoptosis inhibitor as well as an antioxidant, could offer protection against the acrylamide – induced neurotoxicity. Adult male rats were used in the study, divided into 8 groups: a normal control group receiving saline, acrylamide group (30 mg kg⁻¹, three times a week), a calpeptin group (1 mg kg⁻¹ i.p., three times a week), an NAC group (150 mg kg⁻¹ daily), a taurine group (200 mg kg⁻¹ daily), and three combination groups. During four weeks of treatment, all groups were regularly assessed for symptoms of neurotoxicity by monitoring the body weight, hind limb splay, and paralysis. At the end of exposure, biochemical parameters assessing neurotoxicity, apoptosis, and oxidative stress were all examined in brain, skeletal muscle, and serum tissues. Acrylamide caused significant increase in hind limb splay (p<0.001) and paralysis as assessed by functional scoring (p<0.001) versus the normal control rats. This neurobehavioral toxicity was supported by biochemical indices such as inhibition of brain Na⁺/K⁺ ATPase and inhibition of both serum and muscle creatine kinase activity. Such acrylamide-induced neurotoxicity was abolished by the concurrent administration of calpeptin. Moreover, co-treatment of rats with acrylamide and taurine resulted in partial protection against such acrylamide-evoked neurotoxicity. However, in general, the concomitant administration of acrylamide with the well known antioxidant, N-acetyl-L-cysteine (NAC), failed to offer significant protection against the acrylamide-induced neurobehavioral or biochemical changes. Investigating the role of apoptosis revealed that acrylamide induced a significant (p<0.001) 8-fold enhancement in brain calpain activity with a corresponding increase in DNA fragmentation percent. Both parameters were normalized by the concurrent administration of calpeptin. On the other hand, assessing oxidative stress parameters showed significant (p<0.001) acrylamide-induced inhibition of both brain glutathione peroxidase as well as glutathione reductase activities. However, no effect was observed on the level of lipid peroxidation. Neither NAC nor taurine managed to normalize the activity of these enzymes. In conclusion, the data presented suggest that apoptosis might be a major contributor to acrylamide-induced neurotoxicity, while oxidative stress might only play a minor role in such toxicity.

METABOLIC MECHANISM FOR HEMOTOXICITY OF DAPSONE: *IN VITRO* PROFILING OF CYPs RESPONSIBLE FOR METHEMOGLOBINEMIA & OXIDATIVE STRESS

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Dapsone (4,4'-diaminodiphenylsulfone, DDS) is widely used for treatment of *Pneumocystis carinii* pneumonia, as the principal drug in a multidrug regimen for treatment of leprosy, combination treatment for malaria and also is a rapid acting anti-inflammatory agent. The most prevalent adverse effects of DDS are methemoglobinemia and hemolytic anaemia especially seen in patients with G6PD deficiency. DDS is metabolized by cytochrome P-450 to hydroxylamines, which in turn cause methemoglobinemia and hemolysis. However, during the process of methemoglobin formation, erythrocytes are capable of detoxifying the hydroxylamine to the parent drug, which may either reach to the tissues to exert a therapeutic effect or return to the liver and be re-oxidised in a form of systemic cycling. The hemotoxic effects of DDS-NOH may be characterized by significant formation of methemoglobin, accumulation of reactive oxygen intermediates and Heinz body formation. Some additional, still uncharacterized metabolites may also be involved in hematological side effects of DDS. The CYP mediated biotransformation reactions leading to methemoglobin formation and oxidative stress have been studied. An *in vitro* assay, which allows stable as well as unstable metabolites generated *in situ* to react with human erythrocytes, has been employed. Pooled human/mouse liver microsomes and recombinant human CYPs were tested to profile CYPs responsible for methemoglobinemia and oxidative stress. In contrast to 2C9 and 3A4 which predominantly metabolize DDS to DDS-NOH, metabolism of DDS by 2C19 caused highest methemoglobin toxicity. CYP 2B6 and 2D6 also contributed to methemoglobin toxicity of DDS but to a significantly lesser extent than 2C19. Cimetidine and chloramphenicol, which predominantly inhibit 2C19, completely abolished methemoglobin toxicity of DDS mediated by human liver microsomes or recombinant human 2C19. Earlier *in vivo* studies in rodents have also shown improvement of therapeutic/toxic ratio of DDS by cimetidine. However, DDS in presence of human liver microsomes or the recombinant CYPs did not show consistent generation of oxidative stress in the erythrocytes. DDS-NOH, the predominant toxic metabolite of DDS generated dose dependent methemoglobin toxicity as well as oxidative stress and also caused formation of Heinz bodies. The studies indicate that differential metabolic mechanisms might be responsible for methemoglobinemia and oxidative stress response produced by metabolites of DDS. Characterization of the DDS metabolites generated through different CYPs and their hemotoxic potential would help in understanding the metabolic mechanisms for hemotoxicity of DDS.

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MATURATION OF SEROTONERGIC SIGNALING IN OVINE PULMONARY ARTERIES

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Serotonin (5-hydroxytryptamine, 5-HT) is a potent vasoactive hormone, acting at 5-HT receptors in the vasculature. This biogenic amine is especially active in the pulmonary vasculature where it modulates vascular tone and growth. Chronic hypoxia is a known cause of pulmonary arterial hypertension (PAH) and persistent pulmonary hypertension of the newborn (PPHN). Recent studies suggest that chronic hypoxia produces PAH by modulating serotonin signaling. Other studies have shown increased incidence of pulmonary hypertension with the use of drugs like selective serotonin reuptake inhibitors (SSRIs) and fenfluramine (anorexiant). These findings provide evidence for a role of serotonin signaling in enhanced pulmonary arterial contractility. However, it is not known if serotonin plays a role in pulmonary vasoconstriction present in-utero and if serotonergic signaling undergoes maturational changes. Thus, we tested the general hypothesis that with maturation there are changes in serotonin mediated Ca^{2+} signaling in isolated pulmonary arterial smooth muscle cells (PASMCs) studied by imaging cytosolic Ca^{2+} with fura-2. The results show that a significantly smaller percentage of pulmonary arterial cells from fetal responded to serotonin as compared to adult Ovine PASMCs. Further to this, these changes in Ca^{2+} signaling are unique to serotonin. Both fetal and adult Ovine PASMCs have functional sarcoplasmic reticulum (SR), in that cytosolic Ca^{2+} increases were induced by 10 mM cyclopiazonic acid, a sarcoplasmic-endoplasmic Ca^{2+} ATPase inhibitor. The clearance of calcium from the cytosol across the plasma and SR membranes were also similar in fetal and adult PASMCs. Both adult and fetal cells responded equally to 10-100 mM phenylephrine (PE) indicating that IP_3 signaling pathways are functionally intact. These findings suggest there are maturational changes in serotonergic Ca^{2+} signaling, that may translate into alterations in arterial reactivity.

PRELIMINARY CHARACTERIZATION OF A BRAIN-SPECIFIC, NON-AT1/NON-AT2 ANGIOTENSIN BINDING SITE

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In the course of receptor binding and metabolic studies focusing on proper conditions for angiotensin receptor binding studies in the brain, a non-AT1/non-AT2 angiotensin II (Ang II) binding site was revealed. 125I-Ang II receptor binding studies were carried out in membranes obtained from hypothalamus, cerebral cortex, liver and adrenal of rats. Incubations were 1 hr at 24 C in assay buffer: 5 mM EDTA, 150 mM NaCl, 0.1 mM bacitracin and 50 mM NaPO₄ (pH 7.2), 10 μ M PD123319 and losartan, \pm p-chloromercuribenzoic acid (PCMB), (\pm 3 μ M SI-Ang II for non-specific binding). The presence of PCMB (0.1 - 5 mM) was critical for observation of the non-AT1/non-AT2 binding site. At equilibrium binding conditions, 0.3 mM PCMB gave optimal high affinity, saturable binding of 125I-Ang II in the presence of PD123319 & losartan (KD=4.36 \pm 0.78 nM, BMAX=2.07 \pm 0.21 fmol/mg initial wet weight in hypothalamus, n=9; and KD=4.24 \pm 0.89 nM, BMAX=2.72 \pm 0.3 fmol/mg initial wet weight in cerebral cortex, n=11). The BMAX for this binding site was approx. 5 times higher than that for AT1 receptors in the hypothalamus. No specific binding of 125I-Ang II was observed in rat liver or adrenals under the same conditions. 0.3 mM PCMB abolished AT1 receptor binding in the hypothalamus, liver and adrenal. 0.3 mM PCMB did not impair 125I-Ang II binding to AT2 receptors in the adrenal, nor did it impair the ability of PD123319 to block AT2 receptor binding. Competition binding analyses with angiotensin and non-angiotensin peptides revealed the following order of binding affinities: Ang III > Ang II > Ang I >> Ang IV > substance P. Ang (4-8), Ang (5-8), Ang (1-7), Ang (1-6), Ang (1-5), bradykinin, LHRH, VIP and neurotensin had low affinity for the binding site (K_i > 10 μ M). Ang (1-4) increased specific binding of 125I-Ang II by 20-25%. A sulfonic acid derivative of PCMB, p-chloromercuriphenylsulphonic acid (PCMPS) also activated the non-AT1/non-AT2 binding site but with much less potency than PCMB. Other sulfhydryl reagents; mersalyl, N-ethylmaleimide and 5,5'-dithiobis (2-nitrobenzoic acid) (0.3-5 mM) or disulfide-reducing agents dithiothreitol (DTT) (0.3-5 mM) and β -mercaptoethanol (β -MET) (5-30 mM) did not activate the binding site. Moreover, the effects of PCMB and PCMPS were reversed by both DTT and β -MET. Characterization of this binding site as an enzyme, transporter, receptor, or simply the result of artificial conditions is currently underway. [Supported by Peptide Radioiodination Service Center of the University of Mississippi]

ALLOSTERIC MODULATION OF METABOTROPIC GLUTAMATE RECEPTOR 5, M1 AND M4 MUSCARINIC RECEPTORS: POTENTIAL THERAPEUTIC DIRECTIONS FOR SCHIZOPHRENIA

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Growing evidence suggests that NMDA receptor (NMDAR) hypofunction may contribute to schizophrenic symptoms. Unfortunately, direct activation of NMDARs is also associated with an increased risk of excitotoxicity, potentially excessive receptor stimulation, robust receptor desensitization and tolerance with chronic administration. An alternative approach to modulate NMDAR function is to activate receptors that indirectly enhance the signaling of NMDARs. Both mGluR5 and M1/M4 muscarinic receptors are candidates for this type of strategy and it is hypothesized that activation of these receptors may be useful in the treatment of schizophrenic symptoms.

Due to the high sequence homology within these receptor families, it has been difficult to develop truly selective compounds. Recently, our laboratory and others have characterized novel approaches for receptor stimulation through the design of highly selective allosteric activators. For mGluR5, a number of allosteric potentiators have now been developed; these compounds have little or no effect on their own but potentiate the activity of the orthosteric agonist, glutamate. *In vitro* studies with several different structural classes of mGluR5 potentiators reveal that these compounds represent a unique strategy to manipulate mGluR5 function. Furthermore, work with *in vivo* rodent models of antipsychotic action indicates that these compounds are effective in models predictive of antipsychotic activity, suggesting that mGluR5 potentiation represents a novel therapeutic strategy in schizophrenia.



In addition, we have now begun to develop and study selective activators of the M1 and M4 muscarinic receptors using high throughput screening approaches and modification of existing compounds. In the case of M1, we have now performed studies with the compound TBPB (1-[1'-(2-Tolyl)-1,4'-bipiperidin-4-yl]-1,3,-dihydro-2H-benzimidazol-2-one), a novel M1-selective allosteric agonist that robustly increases intracellular calcium in cells expressing M1 receptors (EC_{50} of 140nM). TBPB also shows significant, dose-dependent effects in several *in vivo* schizophrenia-related models, such as amphetamine-induced hyperlocomotion, without producing catalepsy or inducing M2/M3 receptor-mediated adverse effects. We have also developed a series of selective allosteric potentiators of the M4 receptor and show that several of these compounds potentiate the effects of orthosteric activation of the receptor by greater than 20 fold, suggesting that this series represents an exciting new set of pharmacological tools for the study of M4 as a potential therapeutic target.

Supported by grants from NIMH and NINDS.

INHIBITION OF RYANODINE RECEPTORS AND L-TYPE Ca^{2+} CHANNELS BY FLA 365 IN CANINE PULMONARY ARTERIAL SMOOTH MUSCLE CELLS

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Ryanodine (RY) is a highly selective ryanodine receptor (RyR) blocker, but RY binding is dependent on RyR opening. In whole-cell studies, RY binding can lock the RyR in an open-conductance state, short-circuiting the sarcoplasmic reticulum (SR). This restricts studies of $InsP_3$ receptor ($InsP_3R$) activity. Other RyR blockers also have non-selective effects that limit their utility. FLA 365 (4-(2-aminopropyl)-3,5-dichloro-N,N-dimethylaniline) blocks RyR elicited Ca^{2+} increases in skeletal and cardiac muscle, yet its actions on smooth muscle are unknown. Canine pulmonary arterial smooth muscle cells (PASMCs) express both RyRs and $InsP_3Rs$; thus, we tested the ability of FLA 365 to block RyR and $InsP_3R$ elicited Ca^{2+} release by imaging fura-2 loaded PASMCs. Acute exposure to 10 mM caffeine, a selective RyR activator, induced Ca^{2+} increases that were significantly reduced by 20 μM FLA, which was reversible. 10-100 μM FLA 365 reduced Ba^{2+} currents through L-type Ca^{2+} channels in patch voltage clamp studies. FLA 365 (20 μM) nor did not significantly reduce the Ca^{2+} rise elicited by 10 μM 5-HT, which activates multiple Ca^{2+} signaling pathways including $InsP_3Rs$ and L-type Ca^{2+} channels. Thus, it does not appear as though FLA 365 has an effect on the functionality of either 5-HT or $InsP_3$ receptors. Thus, FLA 365 is a novel blocker of RyR that will have utility in the study of Ca^{2+} signaling in smooth muscle cell systems.

SIGMA RECEPTORS MODULATE ARTERIAL FUNCTION AND ARTERIAL MYOCYTE EXCITABILITY

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Sigma receptors (σ) are orphan receptors whose function in tissues and cells is poorly understood. Pharmacologically, there are two σ receptor classes, σ_1 and σ_2 , which are expressed in many tissues including brain, liver, heart, and smooth muscle. Earlier reports indicated that σ receptors are expressed in the vasculature and that they modulate cell excitability. Evidence is accumulating that σ receptor activation induces intracellular signaling events by modulating targets that are important to smooth muscle excitability. For example, σ stimulation reduces delayed-rectifier voltage-gated K (K_v) as well as L-type Ca^{2+} channel activity (Ca_L) and releases intracellular Ca^{2+} stores through $InsP_3R$ dependent pathways. Because of mounting evidence for σ modulation of smooth muscle excitation and the expression of K_v , Ca_L , and $InsP_3R$ in smooth muscle, we hypothesize that σ receptor stimulation modifies vascular smooth muscle excitability by modulating calcium and potassium dependent signaling mechanisms. This was examined by measuring mean arterial pressure (MAP) responses in animals, aortic contractility, as well as cytosolic Ca^{2+} and K and Ba currents through Ca_L in isolated arterial smooth muscle cells (ASMCs) during σ receptor stimulation. The putative σ receptor agonist (1 mg/Kg 1,3-di-*o*-tolylguanidine [DTG] i.v.) caused biphasic MAP responses in awake un-anaesthetized rats. MAP transiently increased by 15 ± 5 mmHg from resting levels, which was followed by a more long lived 8 ± 2 mmHg depression in pressure ($n=3$). Depolarization or α -adrenergic induced contractility was reduced substantially by 100-600 μM DTG. The DTG induced reduction in depolarization induced contractility was partially prevented by the putative σ receptor agonist AC 927 (300 μM). In comparison, 10 μM DTG caused cytosolic Ca^{2+} responses in ~85% of the myocytes examined from rat mesenteric and canine and fetal ovine pulmonary arteries, where Ca^{2+} initially increased above the resting level and then decreased below resting levels. In patch-voltage clamp experiments the peak outward K currents and inward Ba currents were significantly and reversibly reduced by 100~300 μM DTG. These experiments confirm that σ receptor activation modulates vascular and myocyte functions and provides evidence that Ca^{2+} signaling and K_v currents are important contributors.

SYNAPTOSOMAL GLUTAMATE (AMPA) RECEPTOR SINGLE CHANNEL ACTIVITY IS POTENTLY MODULATED BY A NEW AMPAKINE DRUG CX-717

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CX-717, a member of the ampakine family of drugs capable of enhancing cognitive performances, was tested for its ability to modulate the single channel properties of synaptic α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Activity dependent modulation of AMPA receptors at the central synapses are critical for synaptic strengthening mechanisms required for learning and memory. We used isolated hippocampal synaptosomes from adult rats reconstituted in lipid bilayers to investigate the modulatory properties of CX-717 on synaptic AMPA receptor channel properties. Addition of 1.0 μ M CX-717 resulted in many fold increases in channel open probability and mean open time. The single channel conductance and the ability of a selective AMPA receptor antagonist SYM 2206 to block the channel activities were not affected. CX-717, when added to the bilayer containing multi-channel bilayer patches resulted in enhanced interactive channel gating of AMPA receptors that resulted in macroscopic currents with long open times. The fact that CX-717 could enhance such gating behavior raises the possibility that in addition to potentiating single channel activity, CX-717 can also enhance postsynaptic currents by interactive gating and thereby contribute to synaptic strengthening process and memory.

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BASE PAIR: PRE-PROFESSIONAL EDUCATION THROUGH A RESEARCH MENTORSHIP INITIATIVE

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Base Pair (<http://basepair.library.umc.edu>) was created in 1992 as a biomedical research mentorship partnership between the Medical Center and the largest public school district in Mississippi, the Jackson Public School District (JPSD). A 97+% African-American, urban district in which 81% of over 31,000 students qualify for the federal free/reduced lunch program, the JPSD sought innovative ways to enhance science education learning using Medical Center resources. Created initially by the Medical Center Dept. of Pharmacology & Toxicology and supported actively by leadership from that department, *Base Pair* activities have resulted in 124 scientific abstracts or publications, co-authored or presented by high school students in professional scientific forums. Of 131 students, 57% have been African-American, and 63% have been women. Graduates have a 100% continuation rate into a college experience and of 94 who have declared an undergraduate major, 72% have chosen a science field. Fifty-two have completed undergraduate training, of whom 46 have either enrolled in graduate training or have entered a science-related career. Eighteen are enrolled in or have completed a Ph.D., M.D. or an M.D./Ph.D. training program, while 10 others have taken a Masters degree track and 4 have graduated from law school. Six former *Base Pair* students are in or have completed Medical Center medicine, nursing or graduate programs. Mentor-based *Base Pair* training has reached 44 teachers. Professionalism has been advanced by increased publication (51 teacher co-authored abstracts/presentations), acquisition of external funding (63 teacher-initiated applications submitted between 2004-2006, with a 92% funding rate and \$231,418 in additional funds brought into local classrooms), and creation, use and dissemination of novel, inquiry-based learning activities (through local, regional and national meeting presentations, an estimated 975 teachers and 338,000 students have been reached). Extension activities include school-based Student Oriented Academic Research (SOAR) and Rural Biomedical Initiative (RBI) components. Professionalism in science education and science career orientation can be fostered by individual, laboratory-based research mentorship with high school participants. (Supported by the Howard Hughes Medical Institute)

EFFECT OF JWB1-84-1, A NOVEL ANALOG OF CHOLINE, ON DELAYED MATCHING ACCURACY BY AGED RHESUS MONKEYS AND COGNITIVE IMPROVEMENT IN TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

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Choline has been described as a full, but low potency agonist at the $\alpha 7$ subtype of nicotinic acetylcholine receptors. This pharmacological property has been associated with the ability of nicotine and other related $\alpha 7$ receptor agonists to improve working memory in a variety of rodent and non-human primate models. We synthesized over 50 analogs of choline that were shown to have varying abilities to offer cytoprotection in a neural-like cell culture assay. Each compound belonged to one of 4 main structural classes: non-substituted or hydroxyethyl-substituted pyrrolidines; piperazines; or hydroxyethyl-substituted phenyl derivatives. JWB1-84-1, a member of the latter class, was shown to be approximately equipotent with nicotine in the cytoprotection assay. The compound was evaluated for efficacy as a cognition enhancing agent in aged (20-32 y) Rhesus monkeys (4 males, 7 females) who were well trained in the performance of a delayed matching-to-sample task. Touch-sensitive screen/pellet dispenser units were attached to the home cages. A trial was initiated by presentation of a sample rectangle colored red, blue, or yellow. The sample rectangle remained in view until the monkey touched within its borders to initiate a pre-programmed delay (retention) interval. Following the delay interval, the two choice rectangles were presented below and to the right and left of the sample. A correct (matching) choice was reinforced. Errors were not corrected or punished. The inter-trial interval was 5 sec and each session consisted of 96 trials. Vehicle (normal saline) or JWB1-84-1 (5-150 μ g/kg, i.m.) was administered 10 min before initiating testing. On average, JWB1-84-1 treatment improved task accuracy for all but the lowest dose. The maximal degree of improvement, which proved to be significantly different from vehicle-associated accuracies, was attained with the 100 μ g/kg dose. The drug's effects were relegated primarily to Medium and Long delay trials - the most difficult portions of the task, which were improved by up to 18% of control. This dose also improved the number of trials completed such that each subject completed all 96 trials. JWB 1-84-1 treated (2 μ g/kg-50 μ g/kg) mice showed improved performance in Radial Arm Water Maze (RAWM) when compared to saline treated controls. These mice express amyloid plaques in brain at 7 months of age. Improvement in RAWM task was noticed at doses 10 μ g/kg-50 μ g/kg. JWB1-84-1 exhibits potential for both cognition enhancement and neuroprotection and as such the drug could be a good candidate for the treatment of neurodegenerative and cognitive diseases.



SYNTHETIC FIRE ANT VENOM ALKALOIDS: EFFECTS ON HUMAN CELLS

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The effects of four racemic mixtures of synthetic alkaloids, Solenopsins A (Sol A) and B (Sol B) and Isosolenopsins A (Iso A) and B (Iso B), on the human monocytic cell line U937 were examined to determine whether they are toxic for human cells. Viability was determined by staining with trypan blue. Briefly, 1×10^6 U937 cells were plated in triplicate for six different concentrations (3, 10, 13, 20, 23, and 30 μM) of each solenopsin tested. For Sol A, at 48 hours cell viability is reduced by >40% at concentrations of 20 μM and above. Iso A appears to be less toxic, with cell viability reduced at the 48 hour time point by >40% at 30 μM . The most toxic of the compounds was Sol B. At 4 hours, cell viability was decreased >70% at 30 μM Sol B. By 24 hours, cell viability was reduced >50% at 13 μM and above and by 48 hours, at 10 μM and above concentrations of Sol B. Iso B was less toxic: >40% reduction for 30 μM at 4 hours, at and above 20 μM at 24 hours, and 13 μM at 48 hours. U937 cells in media alone and in diluent (cyclodextrin) continued to replicate. Sol B, the most toxic compound, was employed in microarray analysis of 15,067 known human genes and ESTs. Sol B, 13 μM (~4 $\mu\text{g/ml}$), was added to triplicate U937 cell cultures and incubated for 1 or 6 hours. RNA was prepared from media/cyclodextrin controls as well as drug treated cultures at 1 and 6 hours. The RNA was converted to cDNA, labeled with Cy3/Cy5 and hybridized to glass microarrays. A number of up regulated and down regulated genes were identified. At one hour post treatment, 661 genes/ESTs were up regulated 1.5 fold compared to control values. If the more stringent value of a 2.0 fold increase is used to define up regulation, 152 genes/ESTs met the criteria. At six hours post treatment, 620 genes/ESTs are increased at 1.5 fold levels and 68 are increases by 2.0 fold or greater. Very few genes were down regulated at either 1 or 6 hours.

EVALUATION OF NOVEL 2(3H)-BENZOXAZOLONES AND 2(3H)-BENZOTHIAZOLONES AGAINST COCAINE-INDUCED BEHAVIOR IN MICE

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Cocaine is a highly addictive substance that is abused worldwide. Cocaine interacts with σ receptors, suggesting that these sites are important for many of its behavioral effects. The goal of the present study was to investigate whether 2(3H)-benzoxazolones and 2(3H)-benzothiazolonones attenuate cocaine-induced behavioral toxicity in mice. Receptor studies showed that these compounds have nanomolar affinities for both σ_1 and σ_2 receptors. In behavioral studies, male, Swiss Webster mice were pretreated with each of the compounds (0.1-10 mg/kg, i.p.), followed 15 minutes later with a convulsive dose of cocaine (70 mg/kg, i.p.). Pretreatment of mice with various doses of both groups of compounds significantly attenuated cocaine-induced convulsions. This protection appears to be mediated through σ receptor antagonism because traditional σ receptor antagonists with high affinity for these receptors and antisense oligos for σ receptors also attenuated the behavioral toxicity of cocaine. Together, the data suggest that these σ compounds can attenuate against cocaine-induced behavioral toxicity.

PRELIMINARY CHARACTERIZATION OF A BRAIN-SPECIFIC, NON-AT₁/NON-AT₂ ANGIOTENSIN BINDING SITE

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In the course of receptor binding and metabolic studies focusing on proper conditions for angiotensin receptor binding studies in the brain, a non-AT₁/non-AT₂ angiotensin II (Ang II) binding site was revealed. ¹²⁵I-Ang II receptor binding studies were carried out in membranes obtained from hypothalamus, cerebral cortex, liver and adrenal of rats. Incubations were 1 hr at 24 C in assay buffer: 5 mM EDTA, 150 mM NaCl, 0.1 mM bacitracin and 50 mM NaPO₄ (pH 7.2), 10 μM PD123319 and losartan, \pm p-chloromercuribenzoic acid (PCMB), (\pm 3 μM SI-Ang II for non-specific binding). The presence of PCMB (0.1 - 5 mM) was critical for observation of the non-AT₁/non-AT₂ binding site. At equilibrium binding conditions, 0.3 mM PCMB gave optimal high affinity, saturable binding of ¹²⁵I-Ang II in the presence of PD123319 & losartan ($K_D=4.36\pm 0.78$ nM, $B_{MAX}=2.07\pm 0.21$ fmol/mg initial wet weight in hypothalamus, n=9; and $K_D=4.24\pm 0.89$ nM, $B_{MAX}=2.72\pm 0.3$ fmol/mg initial wet weight in cerebral cortex, n=11). The B_{MAX} for this binding site was approx. 5 times higher than that for AT₁ receptors in the hypothalamus. No specific binding of ¹²⁵I-Ang II was observed in rat liver or adrenals under the same conditions. 0.3 mM PCMB abolished AT₁ receptor binding in the hypothalamus, liver and adrenal. 0.3 mM PCMB did not impair ¹²⁵I-Ang II binding to AT₂ receptors in the adrenal, nor did it impair the ability of PD123319 to block AT₂ receptor binding. Competition binding analyses with angiotensin and non-angiotensin peptides revealed the following order of binding affinities: Ang III > Ang I > Ang II >> Ang IV > substance P. Ang (4-8), Ang (5-8), Ang (1-7), Ang (1-6), Ang (1-5), bradykinin, LHRH, VIP and neurotensin had low affinity for the binding site (K_i > 10 μM). Ang (1-4) increased specific binding of ¹²⁵I-Ang II by 20-25%. A sulfonic acid derivative of PCMB, p-chloromercuriphenylsulphonic acid (PCMPs) also activated the non-AT₁/non-AT₂ binding site but with much less potency than PCMB. Other sulfhydryl reagents; mersalyl, N-ethylmaleimide and 5,5'-dithiobis (2-nitrobenzoic acid) (0.3-5 mM) or disulfide-reducing agents dithiothreitol (DTT) (0.3-5 mM) and β -mercaptoethanol (β -MET) (5-30 mM) did not activate the binding site. Moreover, the effects of PCMB and PCMPs were reversed by both DTT and β -MET. Characterization of this binding site as an enzyme, transporter, receptor, or simply the result of artificial conditions is currently underway.



BRAIN AT₁ RECEPTOR SPECIFICITY FOR ANGIOTENSIN II AND ANGIOTENSIN III CONGENERS

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The issue of whether both angiotensin II (Ang II) and Ang III, are active peptides in the brain, or if only Ang III is the active peptide in the brain remains unresolved. Studies of Reaux et al. (Trends Endo. Metab. 2001, 12: 157) and others suggest that Ang III is the sole angiotensin peptide in the brain causing pressor and vasopressin-releasing effects. However, experiments with aminopeptidase-resistant Ang II analogs challenge this notion (Kokje et al., Neuroscience Abstracts 2005). To further explore this question, two aminopeptidase-resistant Ang II antagonist peptides were radiolabeled with ¹²⁵I: Sar¹, Ile⁸ Ang II (¹²⁵I-SI Ang II) and N-methyl-L-Asp¹, Ile⁸ Ang II (¹²⁵I-NI Ang II). Sar is devoid of an alpha carbon side chain so SI Ang II partially mimics Ang III (which lacks the Asp¹ of Ang II). N-methyl-L-Asp has the same alpha carbon side chain as Asp¹ of Ang II and so partially mimics Ang II. Membranes prepared from rat and mouse hypothalamus (in the presence of 10 μM PD123319 to block AT₂ receptors) and liver were used to assess the K_D and B_{max} for the two radioligands. ¹²⁵I-SI Ang II bound to both rat and mouse hypothalamus and liver with a higher affinity than ¹²⁵I-NI Ang II (p<0.05). However, ¹²⁵I-NI Ang II bound to rat and mouse hypothalamic membranes with higher affinity than to liver membranes (p<0.05). In contrast, ¹²⁵I-SI Ang II bound to rat liver membranes with higher affinity than to hypothalamic membranes (p<0.05). ¹²⁵I-SI Ang II also tended to bind to mouse liver membranes with higher affinity than to hypothalamic membranes. ¹²⁵I-SI Ang II also bound with a higher B_{max} than ¹²⁵I-NI Ang II to both rat and mouse hypothalamic membranes (p<0.05), but there was no difference in B_{max} between ¹²⁵I-SI Ang II and ¹²⁵I-NI Ang II in liver membranes. These results suggest that Ang III may bind to AT₁ receptors with higher affinity than Ang II in both the brain and liver. In addition, Ang III may bind to a larger population of AT₁ receptors in the hypothalamus than Ang II. However, since ¹²⁵I-NI Ang II binds to hypothalamic membranes with higher affinity than to liver membranes, this suggests that Ang II is an active angiotensin peptide at brain AT₁ receptors.

EFFECTS OF HOODIA GORDONII EXTRACTS ON THE CENTRAL REGULATION OF BLOOD PRESSURE IN RATS

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Hoodia gordonii extract and specifically P57AS3, an oxypregnane glycoside isolated from it, is touted for its anorexigenic properties. Currently, large number of formulations containing *Hoodia* are widely marketed as a dietary supplement to promote weight-loss. *H. gordonii* is a succulent cactus that grows in sub-Saharan Africa that is consumed by its natives as an appetite-suppressant and as an aid in hydration during long hunting expeditions. However, there has been little study of its mechanism(s) of action, side-effects, or safety. Since many anorexic agents affect the sympathetic nervous system, we examined *H. gordonii* extract and P57AS3 for their effects on blood pressure and vascular tone. Adult male rats, implanted with telemetric probes (TA11PA-C40; Data Sciences International, Arden Hills, MN) for monitoring abdominal aortic blood pressure, and with intracerebroventricular guide cannulas, were administered *H. gordonii* extract (40 μg in 2 μL) or P57 fraction (2 μg in 2 μL) intracerebroventricularly. Both *H. gordonii* extract and P57AS3 caused a short latency reductions of blood pressure, -12±2 and -13±4 mm Hg, respectively. In contrast, application of *H. gordonii* extract or P57AS3 to isolated aortas was without effect on resting tension, tension in phenylephrine-precontracted aortas, or in high potassium-precontracted aortas. These observations indicate that extracts of *H. gordonii* and the P57AS3 have direct effects on brain sites that regulate blood pressure. It appears that the P57AS3 as well as other substances in *H. gordonii* extract contribute to the depressor effect. This depressor action in addition to the reported anorexic actions suggests that *H. gordonii* may be of benefit for the treatment of obesity associated with hypertension. It also suggests that *H. gordonii* may contain novel antihypertensive substances of therapeutic significance. This research was supported by The University of Mississippi.

IDENTIFICATION OF NOVEL INHIBITORS OF MALARIAL LACTATE AND MALATE DEHYDRIGENASE AS POTENTIAL ANTIMALARIAL AGENTS

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Malaria is a major parasitic disease, which causes more than 300 million acute illnesses and about two million deaths annually throughout the tropical and sub-tropical regions of the world. Strains of the malaria parasites with multiple resistances to most of the antimalarial drugs such as chloroquine, mefloquine, sulfadoxine, halofantrine, and pyrimethamine are emerging, which urges scientists to develop new classes of antimalarial drugs that aim different targets. The glycolytic pathway is the major source for energy to the parasite and considered a good antimalarial chemotherapeutic target. Lactate Dehydrogenase (P_LLDH), 2-hydroxy acid oxidoreductase, from *Plasmodium falciparum*, the causative agent of malaria, is a key enzyme in the anaerobic glycolytic pathway. Lactate dehydrogenase is involved the final step in glycolysis reducing pyruvate to lactate with the help of NADH, which is converted to NAD⁺. Effective inhibition of LDH may stop production of ATP, followed by parasite death. However, recently we have found that in *P. falciparum* a cytoplasmic, tetrameric α-proteobacterial malate dehydrogenase (P_MMDH) may complement the NAD⁺/NADH coupling function of LDH when the later is inhibited in culture. Striking structural similarities as well as affinity to utilize artificial cofactor (APADH) by P_LLDH and P_MMDH prompted us to develop a strategy for identification of dual inhibitors of LDH/MDH as potential antimalarial candidates. Oxamate is a competitive inhibitor of the binding of pyruvate to LDH and also manifests low binding affinity against P_LLDH. The approach has been to design and synthesize oxamic acid derivatives that bind both pyruvate and cofactor binding sites. The design of derivatives was conducted by constructing virtual libraries employing docking scores as a penultimate filtration step. These oxamic acid derivative compounds were screened against a battery of recombinant (P_LLDH and P_MMDH) as well as purified mammalian enzymes (LDH, cytoplasmic MDH and mitochondrial MDH). Some novel analogs with 12-130 fold selectivity towards the parasite



enzyme have been identified. Some analogs inhibited both *Pf*LDH and *Pf*MDH at sub micromolar concentrations. Kinetic analysis suggested noncompetitive inhibition of the enzyme by these analogs. However, none of the analogs showed significant antimalarial activity in *in vitro P. falciparum* cultures.

NEW INSIGHTS INTO THE MODE OF ACTION OF ARTEMISININ

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Artemisinin, the anti-malarial constituent of the Chinese herb *Artemisia annua*, has fascinated both medicinal chemists and biologists over the last three decades. Research during this period has led to the development of a number of artemisinin derivatives such as artemether, arteether, and artesunate, which are currently in clinical use against malaria; either alone or in combination with other anti-malarials. Results from mechanistic studies indicate that ferrous iron from heme formed as a result of hemoglobin degradation in the food vacuole of malarial parasite interact with peroxide group of artemisinin and generate oxygen radical, which, on intramolecular hydrogen abstraction lead to the formation of carbon centered radicals capable of alkylating various biological targets. Recent studies have also demonstrated that artemisinin is equally reactive towards heme from undigested hemoglobin, which makes the probable site(s) of action of this molecule. Artemisinin targets identified thus far include translationally controlled tumor protein (TCTP), reduced glutathione, heme and a SERCA type calcium ATPase (PfATP6), among which, proof from animal studies exist only in support of artemisinin-heme adduct. Recent studies from our laboratory, as well as by others, have shown the potential of artemisinin class of compounds to target parasites such as leishmania and trypanosoms. Since peroxide group in the molecule was found crucial for these activities, investigations to explore the uptake, localization and probable targets of artemisinin analogs was initiated. The parasite-drug interactions were monitored using fluorescently tagged artemisinin analogs. In *L. donovani* promastigots the dye bound analog was localized in clear isolated pockets. By using organelle specific dyes their presence in nuclei and mitochondria was ruled out while they might be transported to lysosomes and glycosomes. In the human erythrocytes infected with the malarial parasite *P. falciparum* an explicit stage wise transport of the analogs from initial to mature stages of the parasite infected erythrocytes was seen and no artemisinin analogs were taken up by the uninfected erythrocytes. The results provide new insights and direct evidence on the intracellular sites targeted by artemisinin and should help in better understanding of the mechanism of its selective antiparasitic action.

ROLE OF AMINE OXIDASES IN METABOLISM OF 8-AMINOQUINOLINES

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8-Aminoquinolines are important class of anti-infective drugs with promising use for treatment against malaria, leishmaniasis, Chagas disease and *Pneumocystis carinii* Pneumonia (PCP). Primaquine (PQ) has been the only drug available for treatment of relapse cases of malaria, while few other 8-aminoquinolines are currently under development. The racemic 8-[(4-amino-1-methylbutyl)-amino]-5-(3',4'-dichlorophenoxy)-6-methoxy-4-methylquinoline (NPC1161C), originally synthesized at WRAIR, USA has shown excellent *in vivo* oral activity against blood & tissue stages of malaria parasite, *Leishmania donovani* infection in animals and also *Pneumocystis carinii* pneumonia (PCP). NPC1161C was resolved into two enantiomers namely NPC1161A, the (+) enantiomer and NPC 1161B, the (-) enantiomer. The two enantiomers have shown interesting and important differences in their efficacy and toxicity. Biotransformation of 8-aminoquinolines has been considered important for their efficacy as well as toxicity. Carboxymetabolite has been identified as a major plasma metabolite of primaquine. Similarly carboxyNPC1161 is the only metabolite identified during *in vivo* efficacy studies. Initial *in vitro* experiments with pooled human liver microsomes and S9 fraction did not show metabolism of NPC1161. Metabolism of primaquine to carboxyprimaquine was confirmed in these reactions. Both primaquine and NPC1161 were not metabolized by the human erythrocytes. An incubation time dependent decrease in level of parent drug was observed when primaquine or NPC1161 were incubated with the culture medium supplemented with 10% bovine serum. Disappearance of PQ and NPC1161 was due to their conversion to the aldehyde metabolites by amine oxidases present in serum as the carboxymetabolites appeared when the incubation medium was supplemented with purified aldehyde dehydrogenase. Metabolism of PQ and NPC1161 to carboxymetabolites also occurred in isolated human hepatocytes & HepG2 cell cultures. However, metabolism of NPC1161 was considerably slow as compared to PQ. Metabolism of NPC1161B was significantly faster as compared to NPC1161A in serum, hepatocytes and HepG2 cells. Formation of caroxyPQ and carboxyNPC1161 was not inhibited by the inhibitors of cytochrome P-450 and monoamine oxidases (MAO).

EVALUATION OF NEUROPHARMACOLOGICAL PROPERTIES OF SILDENAFIL (VIAGRA®)

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Objective: To investigate the neuropharmacological properties of sildenafil pertaining to Parkinson's disease.

Background: Patients suffering from Parkinson's disease (PD) also suffer from sexual dysfunction and fatigue. Dopamine agonists are used in the treatment of PD and have shown to enhance sexual activity. Sildenafil is widely prescribed oral therapy for erectile dysfunction. Sildenafil can cross the blood-brain barrier, induce neurogenesis and improve learning/ memory. However, the effect of sildenafil on parkinsonian animal model (s) and fatigue are unknown. Similarly, the antioxidant properties, effect on DNA and mitochondrial complex-I activity have not been established.



Material and Methods: C57/Bl6 mice were intraperitoneally injected with sildenafil (10mg/kg) 30 minutes prior to administration of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP, 30mg/kg i.p., twice, 16hr apart). Striatum was dissected out five-days after the last injection and monoaminergic neurotransmitters were analyzed using HPLC-ECD. Lipid peroxide and protein carbonyl content were measured spectrophotometrically and monoamine oxidase activity was measured fluorimetrically. Valid 6-hydroxydopamine lesioned (6-OHDA, unilaterally) rats were used to investigate the dopamine agonistic or releasing effect of sildenafil. 6-OHDA infused rats were administered with sildenafil (1, 2.5, 5 & 10 mg/kg, i.p.) and stereotypic rotational behavior was analyzed using rotameter. To study the effect on fatigue, sildenafil (2.5, 5 & 10mg/kg, i.p.) was administered to C57/Bl6 mice and forced swim test was performed. *In vitro* experiments were conducted to study the effect of sildenafil on mitochondrial complex I activity and DNA fragmentation. Data were analyzed using Sigma-Stat(2.03).

Result: Sildenafil alone had no effect on the monoaminergic neurotransmitters in the nigrostriatal tract. Sildenafil did not protect against MPTP-induced dopamine depletion in striatum. Sildenafil had no effect on the monoamine oxidase activity. Sildenafil did not induce contralateral/ipsilateral rotation in 6-OHDA lesioned rat. Sildenafil treatment did not affect the total swim time. Sildenafil had no effect on complex I activity and did not induce DNA damage.

Conclusion: Sildenafil might not have dopamine agonistic effect or have any effect on dopamine release. Sildenafil failed to exert neuroprotective effect against MPTP-induced toxicity. Sildenafil also did not possess any anti-fatigue or antioxidant properties.

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GLYCEOLLIN I, A NOVEL ANTI-ESTROGENIC PHYTOCHEMICAL ISOLATED FROM SOY

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Background: Interest in the physiologic and pharmacologic role of bioactive compounds present in plants has increased dramatically over the last decade. Of particular interest in relation to human health are the classes of compounds known as the phytoestrogens, which embody several groups of non-steroidal estrogens that are widely distributed within nature and particularly in soy containing foods. We have identified the isoflavonoid glyceollins I, II and III in soy plants grown under stressed conditions which exhibit marked anti-estrogenic effects on ER function. Here we examined the ability of glyceollin I to suppress the proliferation of ER-positive estrogen dependent cancer cells and inhibiting ER-dependent gene expression.

Materials and Methods: The effects of glyceollin I on proliferation were determined by colony assays. ER-dependent cells were plated in 6 well plates, treated with glyceollin I with and without estrogen stimulation. Colony formation was determined after 14 days of incubation. Gene expression was determined by ERE-luciferase and RT-PCR assays. ER-dependent cancer cell lines were transfected with an ERE-Luc plasmid, treated with glyceollin I in the presence and absence of estrogen stimulation, and harvested for luciferase activity. The cells were also analyzed for the expression of PgR and SDF-1 genes after glyceollin treatment with and without estrogen stimulation.

Results: We have established the ability of glyceollin I to significantly suppress proliferation of ER-dependent cancer cells. We further demonstrate that the effects of glyceollins in suppression of cell growth correlate with inhibition of estrogen stimulated gene expression and suppression of ERE-reporter gene activation.

Discussion: Our results establish the *in vitro* inhibition of estrogen-dependent cell growth by glyceollin I and also provide critical information in the understanding of estrogen-related cancers. The glyceollins may represent important components of a soy-based diet in terms of chemoprevention and treatment of estrogen-related cancer.

NOVEL SIGMA RECEPTOR AGONISTS PRODUCE ANTIDEPRESSANT-LIKE EFFECTS IN MICE

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Many antidepressant drugs interact with σ receptors and their activation produces antidepressant like effects. σ Receptors are localized in brain regions that are affected in depression. Therefore, σ receptor agonists were evaluated for antidepressant-like activity in mice using the forced swim test. Mice were injected (i.p.) with a drug or control, and then placed in a cylinder of water. Immobility time was quantified, and reduction in immobility time was used as an indicator of antidepressant-like actions. Desipramine and fluvoxamine, clinically used antidepressants, served as positive controls and were shown to dose-dependently reduce immobility time ($P < 0.01$). The established σ receptor agonist di-o-tolylguanidine also reduced immobility time ($P < 0.05$). Likewise, the novel σ receptor agonists UMB23 and UMB82 produced antidepressant-like actions by reducing immobility time ($P < 0.01$). The antidepressant-like effects of UMB23 and UMB82 were significantly attenuated by the σ receptor antagonist BD1047 ($P < 0.01$), confirming the involvement of σ receptors in the observed effects. Locomotor activity was measured to determine whether stimulant effects could account for apparent antidepressant-like actions in forced swim tests. After the administration of drugs, mice were put into chambers with photocell monitors. Locomotor activity was quantified by continuous automated counting of photobeam interruptions. Data were evaluated corresponding to the data collection period of the forced swim tests. This part of the study showed that changes in locomotor activity could not explain the antidepressant-like actions of the σ receptor agonists in the forced swim tests. Together, the data provide further evidence that σ receptor agonists represent a possible new class of antidepressant medication.



NEUROADAPTATIONS IN FRA-2 AND SIGMA RECEPTOR GENE AND PROTEIN EXPRESSION ARE ASSOCIATED WITH COCAINE-INDUCED SENSITIZATION

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Using a cocaine-induced behavioral sensitization model coupled with gene and protein expression studies in mice, we showed that cocaine, through its interaction with σ_1 receptors, activates a novel intracellular cascade, resulting in persistent changes in gene and protein expression which correspond with altered responding to cocaine. Male, Swiss Webster mice were assigned to one of the following experimental groups: saline+saline, saline+cocaine, BD1063 (σ_1 receptor antagonist)+cocaine, BD1063+saline. On Days 1-5, the mice received their assigned treatments once a day, and then were allowed a 10 day drug free period. On Day 15, all of the mice were challenged with saline+cocaine. Gene and protein measurements were made using real time PCR and Western blots. Cocaine-induced the expression of the immediate early gene fra-2, which led to progressive increases in σ_1 receptor gene and protein expression over a period of days. The progressive increase in σ_1 expression corresponded to the steady increase in the locomotor response to repeated cocaine administration (development of behavioral sensitization). The cocaine-induced changes in fra-2 and σ_1 receptor gene and protein expression occurred in brain regions subserving drug abuse, such as the cortex, striatum and hippocampus, but not the cerebellum. Moreover, the prototypic σ_1 receptor antagonist BD1063 significantly attenuated both the molecular changes and behavioral sensitization induced by cocaine. These data demonstrate that repeated exposure to cocaine elicits molecular adaptations in neuronal signaling pathways which ultimately manifest as altered behavioral responses to cocaine.

SELECTIVE SCREENING OF MARINE NATURAL PRODUCTS AS NOVEL ANTIDEPRESSANT DRUG LEADS

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Depression is a debilitating disorder affecting over 20% of the total population of the United States. Current antidepressant drugs can take up to several weeks before therapeutic effects are noticed. In addition to the slow time for beneficial use, nearly 30% of these patients fail to respond to the drug treatments, thereby increasing the necessity to develop new antidepressant drugs that are more active and selective toward the proteins and brain regions targeted by this disease. Several classes of marine natural products have shown structural similarities to known antidepressant drugs or their targeted neurotransmitter systems. As of now, little research is being done in drug development for neurological disorders using marine natural products. We have identified two classes of marine natural products that may prove to be a source for potential antidepressant drug therapies. In this study, the forced swim test was used to assess for antidepressant-like activity. If the compound was statistically significant in the forced swim test, it then went on to be tested in the locomotor test to determine if the activity found was due to hyperactivity caused by the compound. Two of the marine compounds that were tested, aaptamine and 5,6-dibromo-N,N-dimethyltryptamine, were determined to have significant antidepressant-like activity in mice, but neither drug showed an increase in locomotor behavior, indicating that the decreased immobility time in the forced swim test was not due to hyperactivity from the drug. Because there is little research on using compounds that are derived from marine natural products on neurological disorders, these results show a potential for these marine natural products to be a drug lead for antidepressant medications.

SIGMA (σ) RECEPTOR ANTAGONIST, BD1063, PROTECTS AGAINST METHAMPHETAMINE-INDUCED HYPERTHERMIA AND NEUROTOXICITY IN MICE

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Methamphetamine (METH) is an addictive drug known to produce hyperthermia and neuronal damage. The dopaminergic pathway is particularly sensitive to METH. METH can act as a neurotoxin to dopaminergic neurons when administered at high doses or with repeated use. METH is also known to interact with σ receptors, which are located on dopaminergic neurons. The goal of the present study was to investigate whether the σ receptor antagonist, BD1063, protects against METH-induced hyperthermia and dopamine damage in the mouse brain. Male, Swiss Webster mice were injected (i.p.) every 2 hours for a total of 4 injections with one of the following treatments: saline + saline, saline + METH (5 mg/kg), BD1063 (10, 20, 30 mg/kg) + saline, or BD1063 (10, 20, 30 mg/kg) + METH (5 mg/kg). Core body temperature was measured one hour after each injection. One week after treatment, dopamine levels were measured from the striatum and cerebellum. METH significantly decreased striatal and cerebellar dopamine levels compared to saline controls. BD1063 (10-30 mg/kg) had no effect on its own on dopamine levels. Pretreatment with BD1063 (10-30 mg/kg) significantly attenuated dopamine loss induced by METH (5 mg/kg) in those brain regions studied. The neuroprotection with BD1063 was accompanied by a decrease in METH-induced hyperthermia. These results suggest that the σ receptor antagonist BD1063 protects against METH-induced hyperthermia and dopamine damage in the mouse brain. Additional studies are needed to further define the mechanisms that mediate this protective effect.

AC927, A SIGMA (σ) RECEPTOR ANTAGONIST, ATTENUATES METHAMPHETAMINE-INDUCED HYPERTHERMIA AND DOPAMINE DAMAGE IN MOUSE BRAIN

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At a single dose, methamphetamine is a locomotor stimulant, whereas it can act as a neurotoxin to monoamine neurons when administered at high doses or repeatedly in rodents. Methamphetamine can also interact with sigma (σ) receptors. The goal of the present study was to evaluate the effects of a σ receptor antagonist on methamphetamine-induced hyperthermia and dopamine damage in the mouse brain. Male, Swiss Webster mice were injected (i.p.) every two hours for a total of four injections with one of the following treatments: Saline/Saline, Saline/Methamphetamine (5, 10 mg/kg), AC927 (5, 10, 20 mg/kg)/Saline, or AC927 (5, 10, 20 mg/kg)/Methamphetamine (5, 10 mg/kg). One week after treatment, dopamine levels were measured from the mice striata and cerebella. Methamphetamine significantly decreased striatal and cerebellar dopamine levels compared to saline controls. AC927 (5-20 mg/kg) had no effect on its own on dopamine levels. Pretreatment with AC927 (5-20 mg/kg) significantly attenuated dopamine loss induced by methamphetamine (5 mg/kg) in those brain regions studied. The neuroprotection with AC927 was accompanied by a decrease in methamphetamine-induced hyperthermia. These results suggest that the σ receptor antagonist AC927 protects against methamphetamine-induced hyperthermia and dopamine damage in the mouse brain.

USING AC927 TO FIND A CURE FOR DRUG ABUSE: METHAMPHETAMINE

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Methamphetamine is an addictive stimulant and the need for medications that treat addiction to this drug is becoming an increasingly serious matter. Researchers have found that methamphetamine interacts with sigma receptors. We hypothesize that blocking the interaction between sigma receptors and methamphetamine will lessen the stimulant effects of methamphetamine. Therefore, the goal of this study was to use a selective sigma receptor antagonist, AC927, to block the stimulant effects of methamphetamine. Stimulant effects were measured by monitoring photobeam breaks in an automated locomotor activity chamber and quantifying the ambulatory, fine, and rearing movements of the animals. The study was conducted in three parts to determine the following: 1) dose response curve for methamphetamine, 2) dose response curve of AC927, and 3) AC927 antagonism of methamphetamine. The results were evaluated using analysis of variance (ANOVA). Methamphetamine and AC927 were each shown to alter locomotor activity in a dose-dependent manner. Moreover, when mice were pretreated with a dose of AC927 that alone had no significant effects on locomotor activity, the compound was successful in antagonizing the stimulant effects of methamphetamine. Therefore, it was concluded that AC927 is a potential candidate for the treatment of methamphetamine abuse.

EVALUATION OF NOVEL 2(3H)-BENZOXAZOLONES AND 2(3H)-BENZOTHIAZOLONES AGAINST COCAINE-INDUCED BEHAVIORS IN MICE

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Cocaine is a highly addictive substance that is abused worldwide. Cocaine interacts with σ receptors, suggesting that these sites are important for many of its behavioral effects. The goals of the present study were to validate the interaction of these compounds with σ receptors and to investigate whether 2(3H)-benzoxazolones and 2(3H)-benzothiazolones attenuate cocaine-induced behaviors in mice. Radioligand binding studies showed that these compounds had nanomolar affinities for both σ_1 and σ_2 receptors. In behavioral studies, male, Swiss Webster mice were pretreated with select compounds (0.1-10 mg/kg, i.p.), followed 15 minutes later with a convulsive (70 mg/kg, i.p.) or locomotor stimulatory (20 mg/kg, i.p.) dose of cocaine. Pretreatment of mice with the compounds significantly attenuated cocaine-induced convulsions and locomotor activity. Alone, the compounds did not promote convulsions or alter locomotor activity. The protective effects of the novel compounds are thought to be mediated through σ receptor antagonism because traditional σ receptor antagonists with high affinity for these receptors and antisense oligonucleotides for σ receptors also attenuate the behavioral effects of cocaine. Together, the data suggest that these structural classes of σ compounds have the potential to be further developed as possible treatments for cocaine abuse.

S-ADENOSYL-L-METHIONINE INCREASES METHANOL, FORMALDEHYDE AND FORMIC ACID IN THE RAT BRAIN TISSUE: A POSSIBLE ROLE OF PROTEIN METHYLATION IN SAM-INDUCED PD-LIKE CHANGES

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Recently, an excess methylation hypothesis has been proposed as a precipitating factor for Parkinson's Disease (PD) because S-adenosylmethionine (SAM), the endogenous methyl donor, induced PD-like changes when injected into the rat brain. SAM is involved in various biological processes, including the methylation of biogenic amines, DNA, RNA, lipids and proteins. The carboxymethylation of proteins is of major interest because dopamine receptor proteins are among the regulator proteins that are methylated and the hydrolysis of the protein methyl ester generates methanol. Since methanol is known to be oxidized to formaldehyde and further to formic acid in the body, we investigated the effects of SAM on the production of methanol, formaldehyde and formic acid in rat brain tissue. The results showed that SAM increased the formation of methanol, formaldehyde and formic acid in a concentration and time dependent manner. From concentrations of 168, 334, 668 and 1336 μ M of [³H-methyl]-SAM, we recovered 62.7, 160.6, and 315.6 fmol/mg of [³H] methanol from rat brain tissue. SAM at concentrations of 50, 100, 500 and 1000 μ M also yielded 4.05, 9.9, 23.75 and 43.95 pmol/mg protein/h of formaldehyde in rat brain tissue. Also, SAM at 250, 500 and 100 μ M produced formic acid at 2.21, 2.55 and 2.77 pmol/mg protein/h, respectively. Equilibrium of the reaction was reached in about 4 hours. Moreover, we tested the relative toxicity



of the metabolites and found that formaldehyde among the three toxic metabolites was most toxic to the neuronal PC12 cells in cell culture study, indicating that formaldehyde may play a role in PCM-induced toxic effects on neurons. Formaldehyde induced cytotoxicity involves oxidative stress since vitamin E partially protected formaldehyde-induced toxic effects. 100 μM formaldehyde caused toxicity in PC12 neuronal-like cells by decreasing cell viability by 75.2%, whereas decreasing cell viability by 45.5% in C6 glia cells after 24h incubation, indicating that neuronal cells are more vulnerable to formaldehyde than glia cells. The present study suggests that carboxymethylation of protein might be involved in SAM-induced PD-like changes, and cause detrimental effects on the brain tissue, possibly via formaldehyde toxicity.

EXPLORING THE ROLE OF 3-O-METHYLDOPA IN THE SIDE EFFECTS OF L-DOPA USING PC12 CELLS

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Parkinson's disease (PD) is a slowly progressing neurological disorder resulting from a deficiency of dopamine in the nigrostriatal pathway due to degeneration of dopaminergic neurons. Since the exact pathogenesis of PD has not been established, the major therapeutic drug for PD is L-3,4-Dihydroxyphenylalanine (L-DOPA). L-DOPA is a precursor of dopamine and it increases the level of dopamine in the brain of PD patients. However, long-term treatment of L-DOPA induces side effects including dyskinesias, wearing off and on-off phenomenon which worsens with continued treatment. It has been proposed that 3-O-methyl DOPA (3-OMD) might be involved in those side effects because 3-OMD is a main metabolite of L-DOPA and accumulates in the brain due to a long half-life (15 h). Cell culture study has been performed using pheochromocytoma cell line (PC12) which is a catecholamine-producing neuronal cell. Cell viability and the generation of reactive oxygen species (ROS) were measured. The results revealed that 3-OMD induced cytotoxic effects and produced ROS, indicating that 3-OMD could damage neuronal cells. Furthermore, 3-OMD potentiates L-DOPA toxicity by increasing ROS formation in PC12 cells. L-DOPA at 62.5 μM and 125 μM increased cytotoxicity in the presence of 3-OMD by 13% and 22%, respectively. L-DOPA at 100 μM increased ROS formation with 1 mM of 3-OMD by 12.5%. Addition of vitamin E (α -tocopherol) into media containing L-DOPA 125 μM and 3-OMD 1 mM completely protected cytotoxicity of PC12 cells, indicating oxidative stress is involved in the toxic effects of L-DOPA and 3-OMD. Therefore, the present study reveals that 3-OMD accumulation from long-term L-DOPA treatment could be involved in L-DOPA induced side effects by damaging dopaminergic neurons. Moreover, our results show that L-DOPA treatment can accelerate the progression of PD at least in part by 3-OMD.

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BRAIN AT₁ RECEPTOR SPECIFICITY FOR ANGIOTENSIN II AND ANGIOTENSIN III CONGENERS

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The hypertensive effects of angiotensin II (Ang II) arise in part from its actions on brain centers that regulate blood pressure. To successfully treat hypertension, it is critical to understand the mechanisms of action of Ang II in the brain. There is good agreement that the AT-1 subtype of Ang II receptors mediates the cardiovascular actions of Ang II, but recent studies, Reaux-Le Goazigo et al. (Curr. Hypertens. Rep. 2005 7:128) suggest that Ang III (des Asp¹ Ang II) rather than Ang II is the active angiotensin peptide in the brain. To address this issue, analogs of Ang II with modified amino acids in position one (or position zero) of the angiotensin peptide, which confer resistance to aminopeptidase activity have been administered ICV into rat brains and their effects have been compared to Ang II and Ang III. These analogs have varying similarity to the Asp¹ amino acid of Ang II. For the most part, the pressor, dipsogenic and salt appetite-inducing effects of the aminopeptidase-resistant Ang II analogs equal or exceed those of Ang II and Ang III. Moreover, the latency of the pressor responses to the aminopeptidase resistant analogs is equivalent or shorter than that of Ang II and Ang III. The duration of the pressor response, and the retention of the thirst response were greater for Ang II and the aminopeptidase-resistant Ang II analogs than for Ang III. Analysis of the rate of metabolism of ICV-administered ¹²⁵I-Ang II versus ¹²⁵I-N-methyl L-Asp¹ Ang II indicated that at the time of the occurrence of a dipsogenic response, no intact ¹²⁵I-Ang II or derived ¹²⁵I-Ang III could be observed in the rat brain, while a small proportion of the ¹²⁵I-N-methyl L-Asp¹ Ang II, but no derived ¹²⁵I-Ang III could be observed upon initiation of the dipsogenic response. Thus these studies indicate that Ang II as well as Ang III are active angiotensin peptides in the brain. Supported by American Heart Association Grant in Aid: 0350481Z and the Peptide Radioiodination Service Center of the University of Mississippi.

KAPPA RECEPTOR MEDIATION OF BUTORPHANOL-INDUCED NEURONAL ACTIVATION WITHIN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS AND RESULTING ACTIVATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

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Previous studies have shown acute peripheral administration of the mixed opioid agonist/antagonist, butorphanol, results in neuronal activation within the PVN. However, these studies did not explore the dose-response relationship between peripheral butorphanol administration and neuronal activation within the PVN. Therefore, the present study employs a rodent model to determine the *in vivo* dose-response relationship between intravenous (i.v.) butorphanol administration and neuronal activation within the PVN and resulting activation of the HPA axis. In addition to dose-response relationships, the role of the kappa opioid receptor (KOR) subtype in butorphanol-induced neuronal activity within the PVN was explored. To determine the dose-effect relationship between butorphanol and PVN neuronal activity, butorphanol (0.1, 1.0, or 10.0 mg/kg; i.v.) was administered and neuronal activity assessed by c-Fos



immunoreactivity. HPA axis activity was assessed via plasma corticosterone concentration. Administration of butorphanol resulted in significant, dose-related increases the number c-Fos immunoreactive cells, percentage of c-Fos immunopositive cell area, and plasma corticosterone levels compared to vehicle. Increases in c-Fos expression within the PVN were positively correlated with increases in plasma corticosterone. The role of the KOR in butorphanol-induced PVN neuronal activation was explored by intracerebroventricular (i.c.v.) administration of nor-binaltorphimine (nor-BNI) prior to butorphanol (10 mg/kg, i.v.) administration. Nor-BNI pretreatment (20 µg) did not reduce either butorphanol-induced c-Fos expression within the PVN or butorphanol-induced plasma corticosterone increases. However, pretreatment with nor-BNI (35 µg) resulted in a significant reduction of c-Fos expression within the PVN of butorphanol treated animals. Our results indicate acute administration of butorphanol elicits dose-related increases in neuronal activity within the PVN resulting in dose related activation of the HPA axis. The KOR appears to mediate butorphanol-induced neuronal activity within the PVN.

NUTRITIONAL COPPER DEFICIENCY PRECIPITATES A SELECTIVE REARRANGEMENT OF VAGAL INPUTS TO THE PANCREAS

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The vagus nerve plays a relevant role in the modulation of pancreatic exocrine and endocrine functions. In animals fed with a copper deficient diet there is a non-inflammatory involution of pancreatic acinar tissue. The purpose of this study was to examine whether the vagal innervation of the pancreas is rearranged following the degeneration of the acinar tissue. Rats, kept on a copper deficient diet since E12, were injected at P20-25 with the anterograde tracer rhodamine-dextran lysine fixable (RD) throughout the rostro-caudal extent of the left dorsal vagal complex. Ten days later, fluorogold (FG) was injected intraperitoneally to label the intrapancreatic ganglia. The pancreatic tissue was extracted three-five days later, weighted and fixed for later analysis. In 3 rats we used the neuronal marker NeuN to label intrapancreatic ganglionic cell bodies. The composition of the pancreas was analysed using NMR and the pattern of vagal innervation was analysed with confocal microscopy. The total weight and the % of pancreatic acinar tissue from copper-deficient animals were significantly reduced compared to control. In control animals the vast majority of FG positive ganglionic cells received putative contacts from RD positive fibers which appear to form baskets around the individual FG positive cells. The ganglia from copper-deficient rats had fewer cells and the RD labeled vagal fiber terminals appear to form bundles around the empty spaces. Our data suggest that the acinar tissue degeneration observed following a copper deficient diet might also induce degeneration of intrapancreatic neurons and a consequent rearrangement of vagal fiber terminals.

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CACO-2 AND MDR-MDCK CELL MODELS TO STUDY BIOAVAILABILITY AND BBB TRANSPORT OF PHARMACOLOGICALLY ACTIVE MOLECULES

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To assess the absorption potential and Blood Brain Barrier (BBB) transport of new chemical entities numerous *in vitro* and *in vivo* models have been used. For intestinal cell permeability assays Caco-2, MDCK, HT-29 and TC-7 cell models have been used. For BBB transport, BBEC, HPBEC, SV 40, MBEC4, MDCK and MDR-MDCK cell models have been reported. Physicochemical methods such as immobilized artificial membrane (IAM) columns and parallel artificial membrane permeability assay (PAMPA) have also been used. Accurate methods are needed to study the absorption and BBB transport properties of lead compounds and to understand their mechanism (efflux-limited absorption, carrier mediated, intestinal metabolism) in an early stage of drug development. In recent years, Caco-2 and MDR-MDCK cell models have been utilized widely and are well accepted due to their close correlation with the *in vivo* systems.

METABOLIC MECHANISM FOR HEMOTOXICITY OF DAPSONE: *IN VITRO* PROFILING OF CYPS RESPONSIBLE FOR METHEMOGLOBINEMIA & OXIDATIVE STRESS*

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Dapsone (4,4'-diaminodiphenylsulfone, DDS) is widely used for treatment of *Pneumocystis carinii* pneumonia, as the principal drug in a multidrug regimen for treatment of leprosy, combination treatment for malaria and also is a rapid acting anti-inflammatory agent. The most prevalent adverse effects of DDS are methemoglobinemia and hemolytic anaemia especially seen in patients with G6PD deficiency. DDS is metabolized by cytochrome P-450 to hydroxylamines, which in turn cause methemoglobinemia and hemolysis. However, during the process of methemoglobin formation, erythrocytes are capable of detoxifying the hydroxylamine to the parent drug, which may either reach to the tissues to exert a therapeutic effect or return to the liver and be re-oxidised in a form of systemic cycling. The hemotoxic effects of DDS-NOH may be characterized by significant formation of methemoglobin, accumulation of reactive oxygen intermediates and Heinz body formation. Some additional, still uncharacterized metabolites may also be involved in hematological side effects of DDS. The CYP mediated biotransformation reactions leading to methemoglobin formation and oxidative stress have been studied. An *in vitro* assay, which allows stable as well as unstable metabolites generated *in situ* to react with human erythrocytes, has been employed. Pooled human/mouse liver microsomes and recombinant human CYPs were tested to profile CYPs responsible



methemoglobinemia and oxidative stress. In contrast to 2C9 and 3A4 which predominantly metabolize DDS to DDS-NOH, metabolism of DDS by 2C19 caused highest methemoglobin toxicity. CYP 2B6 and 2D6 also contributed to methemoglobin toxicity of DDS but to a significantly lesser extent than 2C19. Cimetidine and chloramphenicol, which predominantly inhibit 2C19, completely abolished methemoglobin toxicity of DDS mediated by human liver microsomes or recombinant human 2C19. Earlier *in vivo* studies in rodents have also shown improvement of therapeutic/toxic ratio of DDS by cimetidine. However, DDS in presence of human liver microsomes or the recombinants CYPs did not show consistent generation of oxidative stress in the erythrocytes. DDS-NOH, the predominant toxic metabolite of DDS generated dose dependent methemoglobin toxicity as well as oxidative stress and also caused formation of Heinz bodies. The studies indicate that differential metabolic mechanisms might be responsible for methemoglobinemia and oxidative stress response produced by metabolites of DDS. Characterization of the DDS metabolites generated through different CYPs and their hemotoxic potential would help in understanding the metabolic mechanisms for hemotoxicity of DDS.

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A CELL BASED ASSAY FOR DETERMINING ANTIOXIDANT ACTIVITY OF NATURAL PRODUCTS

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A cell based assay is utilized to screen the antioxidant activity of extracts and pure compounds isolated from medicinal plants. DCFH-DA is used as the fluorescent probe that diffuses into the cells. DCFH-DA is hydrolyzed to DCFH by cellular hydrolases which is converted to DCF as a result of intracellular ROS generation due to oxidative stress. Ability of test samples to inhibit ROS generation is determined in terms of % DCF produced. Vitamin C and Trolox are used as standard antioxidants.

IDENTIFICATION OF NOVEL INHIBITORS OF MALARIAL LACTATE AND MALATE DEHYDRIGENASE AS POTENTIAL ANTIMALARIAL AGENTS

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Malaria is a major parasitic disease, which causes more than 300 million acute illnesses and about two million deaths annually throughout the tropical and sub-tropical regions of the world. Strains of the malaria parasites with multiple resistances to most of the antimalarial drugs such as chloroquine, mefloquine, sulfadoxine, halofantrine, and pyrimethamine are emerging, which urges scientists to develop new classes of antimalarial drugs that aim different targets. The glycolytic pathway is the major source for energy to the parasite and considered a good antimalarial chemotherapeutic target. Lactate Dehydrogenase (*Pf*LDH), 2-hydroxy acid oxidoreductase, from *Plasmodium falciparum*, the causative agent of malaria, is a key enzyme in the anaerobic glycolytic pathway. Lactate dehydrogenase is involved the final step in glycolysis reducing pyruvate to lactate with the help of NADH, which is converted to NAD⁺. Effective inhibition of LDH may stop production of ATP, followed by parasite death. However, recently we have found that in *P. falciparum* a cytoplasmic, tetrameric α -proteobacterial malate dehydrogenase (*Pf*MDH) may complement the NAD⁺/NADH coupling function of LDH when the later is inhibited in culture. Striking structural similarities as well as affinity to utilize artificial cofactor (APADH) by *Pf*LDH and *Pf*MDH prompted us to develop a strategy for identification of dual inhibitors of LDH/MDH as potential antimalarial candidates. Oxamate is a competitive inhibitor of the binding of pyruvate to LDH and also manifests low binding affinity against *Pf*LDH. The approach has been to design and synthesize oxamic acid derivatives that bind both pyruvate and cofactor binding sites. The design of derivatives was conducted by constructing virtual libraries employing docking scores as a penultimate filtration step. These oxamic acid derivative compounds were screened against a battery of recombinant (*Pf*LDH and *Pf*MDH) as well as purified mammalian enzymes (LDH, cytoplasmic MDH and mitochondrial MDH). Some novel analogs with 12-130 fold selectivity towards the parasite enzyme have been identified. Some analogs inhibited both *Pf*LDH and *Pf*MDH at sub micromolar concentrations. Kinetic analysis suggested noncompetitive inhibition of the enzyme by these analogs. However, none of the analogs showed significant antimalarial activity in *in vitro P. falciparum* cultures.

ROLE OF AMINE OXIDASES IN METABOLISM OF 8-AMINOQUINOLINES*

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8-Aminoquinolines are important class of anti-infective drugs with promising use for treatment against malaria, leishmaniasis, Chagas disease and *Pneumocystis carinii* Pneumonia (PCP). Primaquine (PQ) has been the only drug available for treatment of relapse cases of malaria, while few other 8-aminoquinolines are currently under development. The racemic 8-[(4-amino-1-methylbutyl)-amino]-5-(3',4'-dichlorophenoxy)-6-methoxy-4-methylquinoline] (NPC1161C), originally synthesized at WRAIR, USA has shown excellent *in vivo* oral activity against blood & tissue stages of malaria parasite, *Leishmania donovani* infection in animals and also *Pneumocystis carinii* pneumonia (PCP). NPC1161C was resolved into two enantiomers namely NPC1161A, the (+) enantiomer and NPC 1161B, the (-) enantiomer. The two enantiomers have shown interesting and important differences in their efficacy and toxicity. Biotransformation of 8-aminoquinolines has been considered important for their efficacy as well as toxicity. Carboxymetabolite has been identified as a major plasma metabolite of primaquine Similarly carboxyNPC1161 is the only metabolite identified during *in vivo* efficacy studies. Initial *in vitro*



experiments with pooled human liver microsomes and S9 fraction did not show metabolism of NPC1161. Metabolism of primaquine to carboxyprimaquine was confirmed in these reactions. Both primaquine and NPC1161 were not metabolized by the human erythrocytes. An incubation time dependent decrease in level of parent drug was observed when primaquine or NPC1161 were incubated with the culture medium supplemented with 10% bovine serum. Disappearance of PQ and NPC1161 was due to their conversion to the aldehyde metabolites by amine oxidases present in serum as the carboxymetabolites appeared when the incubation medium was supplemented with purified aldehyde dehydrogenase. Metabolism of PQ and NPC1161 to carboxymetabolites also occurred in isolated human hepatocytes & HepG2 cell cultures. However, metabolism of NPC1161 was considerably slow as compared to PQ. Metabolism of NPC1161B was significantly faster as compared to NPC1161A in serum, hepatocytes and HepG2 cells. Formation of caroxyPQ and caroxyNPC1161 was not inhibited by the inhibitors of cytochrome P-450 and monoamine oxidases (MAO).

NEW INSIGHTS INTO THE MODE OF ACTION OF ARTEMISININ

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Artemisinin, the anti-malarial constituent of the Chinese herb *Artemisia annua*, has fascinated both medicinal chemists and biologists over the last three decades. Research during this period has led to the development of a number of artemisinin derivatives such as artemether, arteether, and artesunate, which are currently in clinical use against malaria; either alone or in combination with other anti-malarials. Results from mechanistic studies indicate that ferrous iron from heme formed as a result of hemoglobin degradation in the food vacuole of malarial parasite interact with peroxide group of artemisinin and generate oxygen radical, which, on intramolecular hydrogen abstraction lead to the formation of carbon centered radicals capable of alkylating various biological targets. Recent studies have also demonstrated that artemisinin is equally reactive towards heme from undigested hemoglobin, which makes the probable site(s) of action of this molecule. Artemisinin targets identified thus far include translationally controlled tumor protein (TCTP), reduced glutathione, heme and a SERCA type calcium ATPase (PfATP6), among which, proof from animal studies exist only in support of artemisinin-heme adduct. Recent studies from our laboratory, as well as by others, have shown the potential of artemisinin class of compounds to target parasites such as leishmania and trypanosomes. Since peroxide group in the molecule was found crucial for these activities, investigations to explore the uptake, localization and probable targets of artemisinin analogs was initiated. The parasite-drug interactions were monitored using fluorescently tagged artemisinin analogs. In *L. donovani* promastigots the dye bound analog was localized in clear isolated pockets. By using organelle specific dyes their presence in nuclei and mitochondria was ruled out while they might be transported to lysosomes and glycosomes. In the human erythrocytes infected with the malarial parasite *P. falciparum* an explicit stage wise transport of the analogs from initial to mature stages of the parasite infected erythrocytes was seen and no artemisinin analogs were taken up by the uninfected erythrocytes. The results provide new insights and direct evidence on the intracellular sites targeted by artemisinin and should help in better understanding of the mechanism of its selective antiparasitic action.

