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# Abstracts of Distinguished Lecture and Symposia

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*Distinguished Lecture*

# OXIDATIVE / NITROSATIVE STRESS: IMPLICATIONS FOR CELLULAR DYSFUNCTION

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Free radicals and other reactive species are constantly formed *in vivo* and their levels are controlled by a complex network of endogenous and diet-derived antioxidants. Some oxidative damage occurs even in healthy organisms, making repair systems especially important. Oxidative damage to DNA is thought to contribute to the age-related development of cancer, yet intervention trials with antioxidants have given disappointing results. Our studies on the effects of diet and supplements on levels of oxidative DNA damage in human volunteers may provide an explanation for this discrepancy. By contrast to DNA repair, oxidised proteins are usually degraded by the proteasome. Impairment of proteasome function can thus elevate levels of oxidative damage. This will be explored in detail in my second lecture.

Many reactive species perform useful roles *in vivo*, including nitric oxide and $H_2O_2$. Indeed, exposure of body tissues to $H_2O_2$ may be greater than is commonly supposed. Data on urinary $H_2O_2$ measurements will be presented to support this concept. Nitric oxide in μM amounts can be cytotoxic, in part due to peroxynitrite ($ONOO^-$) formation, often implicated as participating in tissue damage by the detection of 3-nitrotyrosine. However, our work has shown that nitration can occur *in vivo* by multiple mechanisms and must not be taken as a biomarker of $ONOO^-$ formation.

Cell culture studies are often used to study mechanisms of antioxidant action. Yet cells in culture suffer "culture shock", part of which is due to oxidative stress imposed by the cell culture environment and by pro-oxidant effects of cell culture media. Examples of misinterpretations caused by such artifacts in cell culture will be presented.

**References**

1. Beatty, ER, O'Reilly, JD, England, TG, McAnlis, GT, Young, IS, Halliwell B, Geissler, CA, Sanders, TAB and Wiseman, H. (2000) Effect of dietary quercetin on oxidative DNA damage in healthy human subjects. Br. J. Nutr, 84, 919-925.
2. Clement MV, Long LH, Ramalingam J and Halliwell B. (2002) The cytotoxicity of dopamine may be an artefact of cell culture. J. Neurochem. 81, 414-421.
3. Clement, MV, Ramalingam, J, Long, LH and Halliwell B. The in vitro cytotoxicity of ascorbate depends on the culture medium used to perform the assay and involves hydrogen peroxide. Antiox. Redox Signalling, 3, 157-163
4. Halliwell B. (2002) Effect of diet on cancer development. Is oxidative DNA damage a biomarker? Free Rad. Biol. Med. 32, 968-974.

 

5. Halliwell B, Clement MV and Long LH. (2000) Hydrogen peroxide in the human body FEBS Lett, 486, 10-13.
6. Halliwell B. (2000) The antioxidant paradox. Lancet 355, 1179-1180
7. Lee MH, Hyun DH, Marshall KA, Ellerby LM, Bredesen DE, Jenner P. and Halliwell B. (2002) Effect of overexpression of BCL-2 on cellular oxidative damage, nitric oxide production, antioxidant defenses and the proteasome. Free Rad. Biol. Med, 31, 1550-1559.
8. Long LH, Evans PJ and Halliwell B. Hydrogen peroxide in human urine: implications for antioxidant defense and redox regulation. Biochem. Biophys. Res.Commun. 262, 605-609.
9. Long LH and Halliwell B. (2000) Coffee drinking increases levels of urinary hydrogen peroxide detected in healthy human subjects. Free Rad. Res. 32, 463-467.
10. Long LH and Halliwell B. (2001) Oxidation and Generation of Hydrogen Peroxide by thiol compounds in commonly used cell culture media. Biochem. Biophys. Res. Commun. 286, 991–994.
11. Long, LH, Clement, MV, and Halliwell B. (2000) Artifacts in cell culture: Rapid generation of hydrogen peroxide on addition of (-) – epigallocatechin, (-) – epigallocatechin gallate, (+) – catechin and quercetin to commonly used cell culture media. Biochem. Biophys. Res. Commun. 273, 50-53.
12. O'Reilly, JD, Mallet, AI, McAnlis, GT, Young IS, Halliwell B, Sanders, TAB and Wiseman, H. (2001) Consumption of flavonoids in onions and black tea; Lack of effect on $F_2$-isoprostanes and autoantibodies to oxidized LDL in healthy humans. Am. J. Clin. Nutr. 73, 1040-1044
13. Whiteman M, Hooper DC, Scott GS, Koprowski H and Halliwell B. (2002) Inhibition of hypochlorous acid-induced cellular toxicity by nitrite. Proc. Natl. Acad. Sci. USA. 99, 12061-12066.
14. Halliwell B. (2000) Why and how should we measure oxidative DNA damage in nutritional studies? How far have we come? Am.J.Clin.Nutr. 72, 1082-1087
15. Zhao K, Whiteman M, Spencer J and Halliwell B. (2002) DNA damage by nitrite and peroxynitrite: protection by dietary phenols. Meth. Enzymol 335, 296-307.

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*Abstracts of Symposium*

# ROLES OF PINEAL NEUROTRANSMITTERS AND NEUROPEPTIDES

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## Introduction

Pineal gland, in human resembling the shape of a pinecone, is an appendage of the brain. Its location is either central in the brain, close to the third ventricle (humans), or superficial, mostly close to the cerebellum (rat). In all cases, it has a stalk connection (deep pineal) to the habenular commissure. The gland is a small secretory organ. In mammals, the major cellular component of the pineal is pinealocyte. In most species, the pinealocytes are the sites for melatonin synthesis. The major neural innervation is sympathetic fibers, originating from the superior cervical ganglion. This pathway originates in the suprachiasmatic nuclei, which receives light information from the retina through the retinohypothalamic tract. It passes the paraventricular nucleus, follows the medial forebrain bundle and the reticular formation their projection on the superior cervical ganglion exists, from which sympathetic neuron innervates the pineal. The main neurotransmitter is norepinephrine released into the perivascular space, near the pinealocytes.

In addition to receiving adrenergic innervation derived from superior cervical ganglia, the mammalian pineal gland receives a number of other connections from the central and peripheral pinealopetal pathways (28,33). In addition to norepinephrine, the synthesis of melatonin is influenced by other biogenic amines-, amino acids- and peptide transmitters (11,12,13).

Physiological functions of pineal hormone, melatonin

The proposed as well as the established functions attributed to the pineal glands are so extensive and so diversified in nature. Increasing evidence gathered by several laboratories supports that melatonin may contribute in bringing forth the full expression of pineal functions. The multiple functions of this "ubiquitously acting gland have been summarized (13).

Studies over the past decades have shown that melatonin, the pineal secretory product, has a modulatory function in variety systems. The interaction between this ubiquitously acting hormone and a wide range of physiological processes such as the neuroendocrine role, immune system, circadian rhythm, aging process etc.

## Opioidergic innervation of pineal gland

The presence of opioid peptides derived from their precursors, namely; pro-opiomelanocortin, pro-enkephalin, and pro-dynorphin has been detected in mammalian pineal glands by radioimmunoassay. Moreover, by utilizing immunohistochemical techniques, the presence of opioidergic nerve fibers has been demonstrated in the pineal glands of different species including humans (35), cow (31) and tree shrew (37).

An interaction among melatonin, opioids, and analgesia has been suspected for many years, since during nighttime, when the level of melatonin is high, the mammals are less sensitive to pain. In studying this phenomenon further, Govitrapong et al. (20) has identified a single population of opioid receptors in the bovine pineal gland using [$^3$H]diprenorphine and other ligands. It was further

 

demonstrated that opioid receptors are located on the pinealocytes (21). By using reverse transcriptase polymerase chain reaction (RT-PCR) segments of delta and mu opioid receptors were amplified from the mRNA of rat and human pineal glands (6). More recently, we (22) have attempted to identify and characterize the nature and types of opioid receptor in bovine pinealocyte membranes, using a radioligand binding technique with the selective radioligands [$^2$H]DAMGO, [$^3$H]DPDPE, [$^3$H]U69593 and [$^3$H]orphanin-FQ (OFQ) for identifying mu-, delta-, kappa- and opioid receptor-like ($ORL_1$) receptors, respectively. The saturation experiments on bovine pinealocyte membranes for [$^3$H]DPDPE binding provided Bmax and Kd values of 553 $\pm$ 24 fmol/mg protein and 1.3 $\pm$ 0.6 nM; and for [$^3$H]DAMGO binding provided Bmax and $K_d$ values of 6.3 $\pm$ 1.3 fmol/mg protein and 1.2 $\pm$ 0.4 nM, respectively. On the other hand, the specific radioligand ([$^3$H]U69593 and [$^3$H]OFQ) bindings of kappa and $ORL_1$ receptors were undetectable in bovine pinealocyte membranes.

**Possible interaction of opioid system on pineal/melatonin functions**

Previous studies have suggested that opioidergic peptides influence the synthesis of melatonin. For example, the subcutaneous injection of des-tyrosine-γ-endorphin increased the melatonin level (16) and morphine stimulated the release of melatonin from the rat pineal gland. In order to elaborate the function of different types of opioid receptors in pineal glands, we (7) used a selective mu-opioid receptor agonist (DAMGO) and a selective delta opioid-receptor agonist (DPDPE) to investigate the activity of N-acetyltransferase activity. The results of this study showed that both DAMGO and DPDPE stimulated N-acetyltransferase activity and increased the level of melatonin in cultured bovine pinealocytes. These stimulatory effects were blocked by naloxone, an opioid receptor antagonist. However, the kappa-opioid receptor agonist U69593 was unable to alter either the activity of N-acetyltransferase or the level of melatonin.

**Analgesic action of melatonin**

In most species, including the human, the circulating levels of melatonin exhibits a pronounced circadian rhythm with serum levels being high at night (43) and very low during day times (4,43). Since the discovery of morphine, physicians and nurses have noted repeatedly that patients under their care complained of less pain at night and analgesics were given less frequently at night (8) Similarly, oncologists have noted that patients with excruciating cancer pain required extra doses of narcotics during daytime (5).

Physiologists have noted that there exists a significant variation in the day-night pain threshold, with an increase in latency during the dark phase of the daily photoperiod. In most species, including the human, the circulating levels of melatonin display a circadian pattern with maximal values occurring at night. Moreover, chronic pain patients with neurogenic or idiopathic pain disorders had a significantly lower melatonin in serum at 02.00 hr (1). Melatonin (20 to 40 mg/kg i.p.) exhibited maximal analgesic effects at late evening (20.00 hr; see 17,32). In addition, pinealectomy abolished the analgesic action of melatonin (29) and the said effect is reversed by administration of melatonin (25). Naloxone (9) or β-funaltrexamine, an irreversible mu opioid receptor antagonist, disrupts the day-night rhythm of nociception of mice (26), and the said effects seem to be mediated by central and not peripheral opioidergic transmission (30).

Melatonin increased the anti-nociceptive effect of indomethacin and enhanced the anti-inflammatory effect of cysteamin in the carrageenan-induce paw edema (15). The synergic analgesia effect of melatonin with morphine and diazepam and the authors suggest the possible involvement of melatonin as an adjunct medicine for pain patients (36). Melatonin produced the antinociceptive effect in a dose-dependent manner by both i.p. and i.c.v. administrations. This effect was antagonized by i.c.v. naloxone injection (44). It is concluded that melatonin has an

analgesic effect at CNS and the effect is related to the central opioid system. Jeong et al (24) found that increased pain threshold by melatonin, was inhibited by the exposure to magnetic fields or opioid antagonist naloxone. The anti nociception of melatonin is mediated via $MT_2$ receptor within CNS (44). Furthermore, Raghavendra & Kulkarni (38) found that melatonin induced reversal of morphine tolerance and dependence. The authors suggested that the agonistic activity of melatonin towards benzodiazepine receptors might partially contributed to the suppression of morphine dependence but not to the reversal of tolerance to the analgesic activity of morphine.
The full analgesic potential of melatonin or melatonin receptors agonists need to be delineated in exhaustive clinical trails. However, it is doubtful that one can simply augment the analgesic efficacy of narcotics by coadministering melatonin, since melatonin, similar to pentazocine, under certain circumstances may possess both analgesic and partial narcotic receptor antagonistic properties, capable of nullifying the action of narcotic analgesics (9,14).

**Dopaminergic neurotransmission in pineal gland**

The presence of a dopaminergic function in the pineal gland was first suspected by Axelrod et al (3,4), who demonstrated that in addition to L-norepinephrine, dopamine also stimulated the production of [$^{14}$C]melatonin in the cultured pineal gland. Furthermore, the subcutaneous administration of L-dopa increased the activity of N-acetyltransferase (NAT), whereas this activation was not seen in rats pretreated with MK-486, an inhibitor of aromatic L-amino acid decarboxylase, suggesting that the L-dopa-mediated stimulation of NAT was due to the formation of a catecholamine. In addition, following superior cervical ganglionectomy, which denervated the peripheral innervation of pineal gland, the L-dopa-mediated stimulation of NAT became potentiated (10). Moreover, studies by Lynch et al. (31) showed that the administration of L-dopa increased the concentration of melatonin, and this effect was potentiated by pretreatment with 6-hydroxyldopamine, which is known to destroy catecholaminergic nerve terminals (41,42).

Our previous study (19) showed that the concentration of dopamine (6 μg/gm wet tissue) exceeded that of norepinephrine (2 μg/gm wet tissue) in bovine pineal glands. Furthermore, studies from our laboratory have shown that the bovine pineal gland possesses dopamine $D_2$-like receptors (18). Further study (40) reported that the bovine pineal gland also possesses $D_1$ dopamine receptors, which were characterized by using [$^3$H]SCH-23390, the selective $D_1$ dopamine receptor antagonist. The expressions of these dopamine receptor subtypes were determined by using a reverse transcriptase-polymerase chain reaction (RT-PCR) technique with specific pairs of primers to amplify $D_1$ and $D_2$ dopamine receptor mRNAs (39). The results indicate that both $D_1$ and $D_2$ dopamine receptor mRNAs are present in the bovine pineal gland.

Furthermore, the dopamine transporter has been recently identified and characterized in the bovine pineal gland by our group (23). ($^3$H)GRB was chosen to identify the nature of dopamine transporter sites in the pineal gland for the first time. Interestingly, the $K_d$ and $B_{max}$ values obtained from bovine pineal were closer to those values obtained from bovine striatum and frontal cortex. In addition, the dopamine transporter site was sodium and chloride dependent. The results indicate that a dopaminergic neurotransmission exists in the bovine pineal whose physiological functions need to be delineated.

**Possible function of dopamine in pineal gland**

The role of dopamine receptors was investigated by studying the effects of selective $D_1$ and $D_2$ dopamine agonists and antagonists on the N-acetyltransferase activity of cultured bovine pinealocytes (39). The data showed that SKF-38393, a selective $D_1$ agonist, enhanced N-acetyltransferase activity and increased melatonin level, and the stimulatory effect was blocked by SCH-23390, a $D_1$ selective antagonist, whereas quinpirole, a selective $D_2$ agonist, inhibited

N-acetyltransferase basal activity and decreased the melatonin basal level. Furthermore the inhibitory effect was blocked by $D_2$ selective antagonists, spiperone, haloperidol and domperidone. The result indicates that the pineal dopamine receptors have a distinct effect on pineal function, they act independently from those of the sympathetic system.

**Role of melatonin on dopaminergic neuron**

Melatonin has been proven to protect neuronal cells from neurotoxin-induced damage in a widespectrum of neuronal culture systems serving as experimental models for the study of Parkinson's disease. The etiology of dopaminergic neuron death in the substantia nigra of Parkinson patients is not known.

Antolin et al (2) found that melatonin prevented nigral cell death and cell damage induced by chronic administration of MPTP measured as number of nigral cells, tyrosine hydroxylase levels and several ultra-structural features. Recent advances suggest that dysfunction of the mitochondrial respiration and generation of free radicals by oxidative catabolism of dopamine is important mediator of neuronal death in Parkinson's disease. MPTP neurotoxicity depends on the monoamine oxidase B (MAO-B)-catalyzed production of MPP+, which is taken up selectively by dopaminergic neurons and concentrated into the mitochondria. MPP+ inhibits the activity of complex I of the electron transport chain leading to production of reactive oxygen species, loss of the mitochondrial membrane death. Khaldy et al (27) found the synergistic effects of melatonin and deprenyl (MAO-B inhibitor) administration in MPTP-induced Parkinson's disease.

MPTP toxicity is based on direct inhibition of complex I of the electron transport chain in the mitochondria by is metabolite MPP+. Inhibition of the said complex has also been reported in the substantia nigra of patients suffering Parkinson's disease. The antioxidant effects of melatonin and its protective effects against the uncoupling of the electron transport chain of several toxins in the mitochondria have been suggested.

Levels of the antioxidant melatonin tend to decrease with age in contrast to the increased incidence of neurodegenerative disease. Aging and neurodegenerative diseases have been proposed as a consequence of the imbalance (physiological or toxin-induced) between oxidant production by the organism and its antioxidant defense system. Other constituents of this antioxidant system have not been found to decrease with age, melatonin being the only one matching this age-related pattern. This points to an increase in free radicals which are endogenously or exogenously produced. The protective effect of melatonin demonstrated in abundant cell culture experiments together with the *in vivo* protection against the acutely 6-OHDA and MPTP-induced cell damage and the chronically-induced damage by MPTP, make melatonin plausible candidate in the prevention of the appearance of these diseases and give a clue to its use as a treatment to avoid disease progression.

Many substrates such as amphetamine or the dopaminergic neurotoxin, l-methyl-4-phenylpyridinium (MPP+), are substrates for the dopamine carriers and can be transported. The dopamine transporter is also a major molecular target for cocaine, an addictive drug. Therefore, interactions of pharmacological agents with dopaminergic transmission reported for the first time in the bovine pineal gland might have profound neurobiological, pathophysiological and pharmacological consequences.

In conclusion, we have attempted to describe the possible functions of dopamine, as an example of several neurotransmitters and the possible functions of opioid, as an example of many peptidergic transmitters in the pineal gland. Both opioid and dopamine regulate the synthesis of melatonin. Melatonin, a lipid-soluble substance, may interact with numerous receptors for amino acids, biogenic amines and peptides and produce its secondary effect as a cohormone or a cotransmitter. Melatonin may interact with different types of radical and produce its secondary

effect as a free radical scavenger. The total functions of pineal gland would be to orchestrate, to synchronize and to refine the multiplicity of biological functions, which make life possible. The levels of melatonin can be enhanced or attenuated by numerous types of neurotransmitters and/or neuropeptides. The research for and discovery of how the pineal, with its apparent omnipotent effects, brings forth these multiple functions may raise the exciting prospect of providing new avenues of treating numerous diseases, thus replacing old treatments which sustain life but diminish its quality.

**References**

1. Almay BG, von Knorring L and Wetterberg L. Melatonin in serum and urine in patients with idiopathic pain syndromes. *Psychiatry Res* 22: 179-191, 1987.
2. Antolin I, Mayo JC, Sainz RM, del Brio Mde L, Herrera F, Martin V and Rodriguez C. Protective effect of melatonin in a chronic experimental model of Parkinson's disease. *Brain Res* 943: 163-173, 2002.
3. Axelrod J, Shein HM and Wurtman RJ. Stimulation of C14-melatonin synthesis from C14-tryptophan by noradrenaline in rat pineal in organ culture. *Proc Natl Acad Sci U S A* 62: 544-549, 1969.
4. Axelrod J. The pineal gland: a neurochemical transducer. *Science* 184: 1341-1348, 1974.
5. Bruera E, Macmillan K, Kuehn N and Miller MJ. Circadian distribution of extra doses of narcotic analgesics in patients with cancer pain: a preliminary report. *Pain* 49: 311-314, 1992.
6. Chetsawang B, Casalotti SO, Phansuwan-Pujito P, Kotchabhakdi N and Govitrapong P. Gene expressions of opioid receptors and G-proteins in pineal glands. *Biochem Biophys Res Commun* 262: 775-780, 1999.
7. Chuchuen U, Govitrapong P, Chetsawang B and Ebadi M. The roles of Mu and Delta opioid recejptors on the synthesis of melatonin in pineal glands. *28th Congress on Socience and Technology of Thailand*: 454, 2002.
8. Closs SJ. An exploratory analysis of nurses' provision of postoperative analgesic drugs. *J Adv Nurs* 15: 42-49, 1990.
9. Datta PC, Sandman CA and Hoehler FK. Attenuation of morphine analgesia by alpha-MSH, MIF-I, melatonin and naloxone in the rat. *Peptides* 3: 433-437, 1982.
10. Deguchi T and Axelrod J. Induction and superinduction of serotonin N-acetyltransferase by adrenergic drugs and denervation in rat pineal organ. *Proc Natl Acad Sci U S A* 69: 2208-2211, 1972.
11. Ebadi M and Govitrapong P. Orphan transmitters and their receptor sites in the pineal gland. *Pineal Res Rev.* 4: 1-54, 1986.
12. Ebadi M and Govitrapong P. Neural pathways and neurotransmitters affecting melatonin synthesis. *J Neural Transm Suppl* 21: 125-155, 1986.
13. Ebadi M, Hexum TD and Pfeiffer RF. The pineal and retinal peptides and their peptides. *Pineal Res Rev* 7: 1-156, 1989.
14. Ebadi M, Govitrapong P, Phansuwan-Pujito P, Nelson F and Reiter RJ. Pineal opioid receptors and analgesic action of melatonin. *J Pineal Res* 24: 193-200., 1998.
15. El-Shenawy SM, Abdel-Salam OM, Baiuomy AR, El-Batran S and Arbid MS. Studies on the anti-inflammatory and anti-nociceptive effects of melatonin in the rat. *Pharmacol Res* 46: 235-243, 2002.
16. Geffard M, Gaffori O, Chauveau J, Muyard JP and Le Moal M. Dramatic increase in pineal melatonin levels in the rat after subcutaneous injection of Des-tyrosine1-gamma-endorphin. *Neurosci Lett* 27: 329-334, 1981.

17. Golombek DA, Escolar E, Burin LJ, De Brito Sanchez MG and Cardinali DP. Time-dependent melatonin analgesia in mice: inhibition by opiate or benzodiazepine antagonism. *Eur J Pharmacol* 194: 25-30, 1991.
18. Govitrapong P, Murrin LC and Ebadi M. Characterization of dopaminergic receptor sites in bovine pineal gland. *J Pineal Res* 1: 215-226, 1984.
19. Govitrapong P, Hama Y, Pfeiffer R and Ebadi M. Status of dopamine in bovine pineal glands and the stimulation of N-acetyltransferase activity by D2-dopaminergic receptor agonists in the rat pineal glands in culture. *J Pineal Res* 6: 17-31, 1989.
20. Govitrapong P, Pariyanonth M and Ebadi M. The presence and actions of opioid receptors in bovine pineal gland. *J Pineal Res* 13: 124-132, 1992.
21. Govitrapong P, Jitaijamjang W, Chetsawang B, Phansuwan-Pujito P and Ebadi M. Existence and function of opioid receptors on mammalian pinealocytes. *J Pineal Res* 24: 201-208, 1998.
22. Govitrapong P, Sawlom S and Ebadi M. The presence of delta and mu-, but not kappa or ORL(1) receptors in bovine pinealocytes. *Brain Res* 951: 23-30, 2002.
23. Govitrapong P, Vilaipun P, and Ebadi M. Identification and characterization of dopamine transporter in bovine pineal gland. *J pineal Res*., 2003 (in press).
24. Jeong JH, Choi KB, Yi BC, Chun CH, Sung KY, Sung JY, Gimm YM, Huh IH and Sohn UD. Effects of extremely low frequency magnetic fields on pain thresholds in mice: roles of melatonin and opioids. *J Auton Pharmacol* 20: 259-264, 2000.
25. John TM, Brown MC, Wideman L and Brown GM. Melatonin replacement nullifies the effect of light-induced functional pinealectomy on nociceptive rhythm in the rat. *Physiol Behav* 55: 735-739, 1994.
26. Kavaliers M. Beta-funaltrexamine disrupts the day-night rhythm of nociception in mice. *Brain Res Bull* 22: 783-785, 1989.
27. Khaldy H, Escames G, Leon J, Bikjdaouene L and Acuna-Castroviejo D. Synergistic effects of melatonin and deprenyl against MPTP-induced mitochondrial damage and DA depletion. *Neurobiol Aging* 24: 491-500, 2003.
28. Korf HW and Moller M. The innervation of the mamalian pineal gland with special reference to central pinealopetal projections. *Pineal Res Rev* 2: 41-86, 1984.
29. Lakin ML, Miller CH, Stott ML and Winters WD. Involvement of the pineal gland and melatonin in murine analgesia. *Life Sci* 29: 2543-2551, 1981.
30. Lowenstein PR, Pereyra EN, Gonzalez Solveyra C and Cardinali DP. Effect of naloxone on the nocturnal rise of rat pineal melatonin content. *Eur J Pharmacol* 98: 261-264, 1984.
31. Lynch HJ, Eng JP and Wurtman RJ. Control of pineal indole biosynthesis by changes in sympathetic tone caused by factors other than environmental lighting. *Proc Natl Acad Sci U S A* 70: 1704-1707, 1973.
32. Martinez-Gomez M, Cruz Y, Salas M, Hudson R and Pacheco P. Assessing pain threshold in the rat: changes with estrus and time of day. *Physiol Behav* 55: 651-657, 1994.
33. Moller M and Mikkelsen JD. Molecular messengers in brain-pineal interactions. *In: Recent advances in cellular and molecular biology*, edited by Wegmann RJ and Wegmann MA., Leuven, Belgium: Peeters Press, 1992, vol. 3, p. 141-147.
34. Moller M, Mikkelsen JD and Phansuwan P. Demonstration of nerve fibers immunoreactive to met-enkephalin and β-endorphin in the bovine pineal gland. *In: Role of melatonin and pineal peptides in neuroimmunomodulation*, edited by Fraschini F and Reiter R. New York: Plenum Press, 1991, p. 15-25.
35. Moore RY and Sibony P. Enkephalin-like immunoreactivity in neurons in the human pineal gland. *Brain Res* 457: 395-398., 1988.

36. Pang CS, Tsang SF and Yang JC. Effects of melatonin, morphine and diazepam on formalin-induced nociception in mice. *Life Sci* 68: 943-951, 2001.
37. Phansuwan-Pujito P, Jitjaijamjang W, Ebadi M, Govitrapong P and Moller M. Opioidergic innervation of the tree shrew pineal gland: an immunohistochemical study. *J Pineal Res* 24: 209-214, 1998.
38. Raghavendra V and Kulkarni SK. Possible mechanisms of action in melatonin reversal of morphine tolerance and dependence in mice. *Eur J Pharmacol* 409: 279-289, 2000.
39. Santanavanich C, Chetsawang B, Ebadi M and Govitrapong P. The effects of D1 and D2-dopamine receptors activation on melatonin synthesis in bovine pinealocytes. *J Pineal Res*., 2003 (in press).
40. Simonneaux V, Murrin LC and Ebadi M. Characterization of D1 dopamine receptors in the bovine pineal gland with [3H]SCH 23390. *J Pharmacol Exp Ther* 253: 214-220, 1990.
41.Tranzer JP and Thoenen H. Electronmicroscopic localization of 5-hydroxydopamine (3,4,5-trihydroxy-phenyl-ethylamine), a new 'false' sympathetic transmitter. *Experientia* 23: 743-745, 1967.
42. Tranzer JP and Thoenen H. An electron microscopic study of selective, acute degeneration of sympathetic nerve terminals after administration of 6-hydroxydopamine. *Experientia* 24: 155-156, 1968.
43. Wetterberg L. Melatonin in humans physiological and clinical studies. *J Neural Transm Suppl*: 289-310, 1978.
44. Yu CX, Zhu CB, Xu SF, Cao XD and Wu GC. Selective MT(2) melatonin receptor antagonist blocks melatonin-induced antinociception in rats. *Neurosci Lett* 282: 161-164, 2000.

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*Abstracts of Symposium*

# ALZHEIMER'S DISEASE AND OXIDATIVE STRESS

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The brain is often said to be prone to oxidative damage for a variety of reasons, including a high content of iron, high $O_2$ uptake per unit mass and the presence of autoxidisable neurotransmitters. Our recent work with dopamine and its oxidation products has questioned the concept that it may be neurotoxic in Parkinson's disease, however.

Brains from subjects with major neurodegenerative diseases often show mitochondrial dysfunction, increased oxidative damage, and the presence of aggregated proteins. We have been pursuing the hypothesis that the latter is partially due to defects in abnormal protein clearance by the proteasome system. Indeed, we find that inhibition of the proteasome causes increased oxidative damage, accumulation of aggregated and nitrated proteins, mitochondrial dysfunction and cell death by apoptosis. Elevated formation of nitric oxide plays a key role in these events. Cells with proteasomal defects that also over-express abnormal proteins are highly-sensitive to low levels of neurotoxins.

Oxidative stress may be implicated in all the major neurodegenerative diseases, but the evidence is strongest for AD, both from post-mortem studies, studies on lipid peroxidation (isoprostane measurement) in patients and examination of the sequence of events in transgenic animals. Thus there is a need for neuroprotective antioxidants. Strategies for assessing neurodegeneration and neuroprotective agents *in vivo* will be presented.

**References**

1. Clement MV, Long LH, Ramalingam J and Halliwell B. (2002) The cytotoxicity of dopamine may be an artefact of cell culture. J. Neurochem. 81, 414-421.
2. Halliwell B and Jenner P. (1998) Impaired clearance of oxidised protein in neurodegenerative diseases. Lancet, 351, 1510.
3. Halliwell B. (2002) Hypothesis: proteasomal dysfunction: a primary event in neurogeneration that leads to nitrative and oxidative stress and subsequent cell death. Ann N Y Acad Sci 962, 182-194.
4. Halliwell B. (2001) Role of free radicals in the neurodegenerative diseases. Therapeutic implications for antioxidant treatment. Drugs & Aging 18, 685 – 716.
5. Hyun DH, Lee MH, Halliwell B and Jenner P.(2002) Proteasomal dysfunction induced by 4-hydroxy-2,3-*trans*-nonenal, an end-product of lipid peroxidation; a mechanism contributing to neurodegeneration? J. Neurochem. 83, 360-370.

 

6. Hyun DH, Lee MH, Hattori N, Kubo SI, Mizuno Y, Halliwell B and Jenner P. (2002) Effect of wild-type or mutant parkin on oxidative damage, nitric oxide, antioxidant defences and the proteasome. J. Biol. Chem. 277, 28572-
7. Lee MH, Hyun DH, Halliwell B and Jenner P. (2001) Effect of overexpression of wild-type and mutant Cu/Zn superoxide dismutases on oxidant stress and cell death induced by $H_2O_2$, 4-hydroxynonenal or serum deprivation. Potentiation of injury by ALS-related mutant superoxide dismutases and protection by bcl-2 J. Neurochem 78, 209-220.
8. Lee MH, Hyun DH, Jenner P and Halliwell B. (2001) Effect of proteasome inhibition on cellular oxidative damage, antioxidant defences and nitric oxide production. J. Neurochem 78, 32-41.
9. McNaught, K St P, Olanow, CW, Halliwell B, Isacson, O and Jenner P.(2001) Failure of the ubiquitin – proteasome system in Parkinson's disease. Nature Reviews in Neuroscience 2, 589 – 594.
10. Nourooz-Zadeh J, Liu EHC, Yhlen B, Anggard EE, and Halliwell B. (1999) $F_4$-Isoprostanes as specific markers of docosahexaenoic acid (DHA) peroxidation in Alzheimer's disease. J. Neurochem. 72, 734-740.
11. Ong WY, Lu XR, Hu CY and and Halliwell B. (2000) Distribution of hydroxynonenal-modified proteins in the kainate-lesioned rat hippocampus: evidence that hydroxynonenal formation precedes neuronal cell death. Free Rad. Biol. Med. 28, 1214-1221.

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*Abstracts of Symposium*

# NEUREGULIN-1B AND ERBB RECEPTORS APPEAR TO PLAY A ROLE IN REMODELING OF MATURE NEURONS IN THE BRAIN

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Growth and differentiation molecules contribute importantly to many biological functions during the development of the brain. For instance, neuregulin (NRG) and its associated erbB receptors are important in the differentiation of the peripheral nervous system, where they direct the differentiation of Schwann cells from neural crest precursors and contribute importantly to the formation of nerve-muscle synapses. Interestingly, they are reactivated in the peripheral nervous system following injury, and they thereby facilitate the interaction between regrowing axons and Schwann cells, direct the regrowing axon to its appropriate target and reassemble the nerve muscle synapse. Recently, these molecules have been identified as important contributors to the development of the central nervous system, and our studies and those of others demonstrate suggest that NRG and the erbB receptors are also important in the interactions between mature neurons and glial cells, especially during remodeling and synaptic transmission.

Our studies demonstrate that NRG-1 and the three erbB subunits that can form functional NRG-1 receptors are present in high quantities in the mature brains of rats, mice and humans, and they are differentially distributed. These anatomical data suggest that this signaling system is important in the mature brain. First, studies by our group and others indicate that in the mature hippocampus both sustained synaptic excitation and neuronal damage increase the expression of NRG and erbB4 receptor subunits. Second, both NRG-1 and erbB4 are overexpressed in glia that surround β-amyloid plaques in the brains of patients with Alzheimer's disease and in a double transgenic murine model of the disease. This accumulation appears to parallel the degree of pathogenesis and may form a nidus for axonal growth that misdirects normal synaptic reorganization in these brains. Third, application of NRG-1 to differentiated hippocampal neurons induces dendritic outgrowth and elaboration and increases the expression of several neurotransmission-related genes. Fourth, *in vivo* microinjection of NRG-1 in the mature hippocampus enhances synaptic transmission in the entorhinodentate pathway, but inhibits synaptic transmission in the entorhino-CA-1 pathway. Together, these data indicate that NRG-1 is a pluripotent signaling system in the mature brain.
NRG-1 and its erbB receptors are highly and differentially expressed in human and rat brain (Chen et al.,1994; Gerecke et al.,2001), suggesting that they may modulate the reorganization of synapses, alter neuronal morphology and change synaptic strength in the adult CNS. Consistent with this hypothesis, Eilam et al. (Eilam et al.,1998), found that the expression of NRG-1 and erbB4 are upregulated in the hippocampal formation following locomotor activity, seizures and the induction

 

of long-term potentiation (LTP). These findings closely mirror the upregulation of BDNF that results from stimulation of hippocampal afferents(Lindefors et al.,1992), seizure activity (Ballarin et al.,1991; Gall and Isackson,1989), and neuronal damage (Hofer and Barde,1988; Oppenheim et al.,1992; Yan et al.,1992; Sendtner et al.,1992).

NRG-1 also has multiple actions on glia that may be involved in adult nervous system responses to injury. Our data demonstrate that in Alzheimer's diseased brain and in a double transgenic mouse model of the disease, reactive microglia and a subpopulation of astrocytes surround β-amyloid plaques, and these glia densely concentrate NRG-1 and erbB4, indicating that these molecules may play an important role in the CNS response to this neurodegenerative disease (Chaudhury et al.,2003). Taken together with other studies, these results indicate that the NRG-1 isoforms and their erbB receptors may play important roles in CNS injury and disease.

NRG-1 is able to accelerate phenotypic development in differentiated neurons. We have differentiated hippocampal neurons from E18 embryos for five days and then cultured them in serum-free media in the presence or absence of 10nM recombinant NRG-1β (Gerecke et al.,2001). In untreated cultures most neurons aggregated together and had short, simple processes. In contrast, in NRG-1β treated cultures, the neurons were evenly dispersed and showed a marked increase in complexity, length and frequency of processes. Further, When differentiated neurons are treated with NRG-1β, they greatly increased arborizations and dendritic contacts with other neurons. All of these effects were present within 24 hours after NRG1β administration and persisted for at least seven days. These data are consistent with the hypothesis that NRG-1 significantly enhances the plasticity of differentiated neurons in the central nervous system and thus may play a significant role in remodeling in the mature brain.

Our recent studies also demonstrate that NRG-1 can modify indeed modify neuronal transmission *in vivo* at both the entorhinal-dentate and the entorhinal-CA1 synapse in the rat hippocampal formation (Roysommuti and Wyss,2003). Microinjection of NRG-1β into the dentate gyrus causes a dose-related increase in the peak slopes of field excitatory postsynaptic potentials (fEPSP) in the dentate gyrus in response to entorhinal cortex stimulation. Further, increasing doses of NRG-1β decrease the latency of this modulatory effect. Injection of the reversible erbB tyrosine kinase inhibitor PD158780 cause a significant reduction in fEPSP slopes and block the effects of a subsequent NRG injections. In contrast, microinjection of NRG-1β into CA-1 has an opposite effect, i.e., it decreases the peak slopes of fEPSP at the entorhinal CA-1 synapse. These *in vivo* electrophysiological data demonstrate that, at least in anesthetized rats, the transmission of electrical information from the entorhinal cortex to the specific areas within of the hippocampal formation can be differentially altered by infusion of NRG-1β. Further, since acute blockade of NRG-1β signaling blunts entorhinal-dentate and enhances entorhinal-CA1 synaptic transmission, these data suggest that the modulatory effects of NRG-1 in the hippocampus are constitutively active. The difference between the NRG-1β effects in the dentate gyrus versus CA1 may reflect differences in the abundance of erbB receptor subtypes in each location or a difference in NRG-1β induced actions on synaptic properties of the neurons involved.

In summary, while NRG/erbB receptor is important in the mature brain. The effects of this signaling cascade on synaptic function and remodeling in the mature brain appear to recapitulate the functions of NRG/erbB receptors in the developing brain, and like other growth hormones, NRGs can directly modify synaptic transmission in the brain. Thus, the NRGs and the erbB receptors

serve as pluripotential signaling molecules, whose roles in the mature CNS may provide clues to therapeutic strategies for a wide range of CNS insults.

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**References**

1. Ballarin M, Ernfors P, Lindefors N and Persson H. Hippocampal damage and kainic acid injection induce a rapid increase in mRNA for BDNF and NGF in the rat brain. *Exp Neurol* 114: 35-43, 1991.
2. Chaudhury AR, Gerecke KM, Wyss JM, Morgan DG, Gordon MN and Carroll SL. Neuregulin-1 and erbB4 immunoreactivity is associated with neuritic plaques in Alzheimer disease brain and in a transgenic model of Alzheimer disease. *J Neuropathol Exp Neurol* 62: 42-54, 2003.
3. Chen MS, Bermingham-McDonogh O, Danehy FT, Jr., Nolan C, Scherer SS, Lucas J, Gwynne D and Marchionni MA. Expression of multiple neuregulin transcripts in postnatal rat brains. *J Comp Neurol* 349: 389-400, 1994.
4. Eilam R, Pinkas-Kramarski R, Ratzkin B, Segal M and Yarden Y. Activity-dependent regulation of Neu differentiation factor/neuregulin expression in rat brain. *Proc Natl Acad Sci USA* 95: 1888-1893, 1998.
5. Gall CM and Isackson PJ. Limbic seizures increase neuronal production of messenger RNA for nerve growth factor. *Science* 245: 758-761, 1989.
6. Gerecke KM, Wyss JM, Karavanova I, Buonanno A and Carroll SL. ErbB transmembrane tyrosine kinase receptors are differentially expressed throughout the adult rat central nervous system. *J Comp Neurol* 2001.
7. Hofer MM and Barde YA. Brain-derived neurotrophic factor prevents neuronal death in vivo. *Nature* 331: 261-262, 1988.
8. Lindefors N, Ernfors P, Falkenberg T and Persson H. Septal cholinergic afferents regulate expression of brain-derived neurotrophic factor and beta-nerve growth factor mRNA in rat hippocampus. *Exp Brain Res* 88: 78-90, 1992.
9. Oppenheim RW, Yin QW, Prevette D and Yan Q. Brain-derived neurotrophic factor rescues developing avian motoneurons from cell death. *Nature* 360: 755-757, 1992.
10. Roysommuti S and Wyss JM. Brain-derived neurotrophic factor plays a significant role in entorhino-dentate synaptic transmission in adult rats. *FASEB J* In Press: 2003.
11. Sendtner M, Holtmann B, Kolbeck R, Thoenen H and Barde YA. Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. *Nature* 360: 757-759, 1992.
12. Yan Q, Elliott J and Snider WD. Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. *Nature* 360: 753-755, 1992.

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# THE 32nd ANNAUL ACADAMIC MEETING OF THE PHYSIOLOGICAL SOCIETY OF THAILAND

# Abstract: oral session

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***O-01***

# THE ROLE OF NITRIC OXIDE, ANGIOTENSIN II AND ENDOTHELIN ON RENAL FUNCTION AFTER ISCHEMIA/REPERFUSION IN RATS

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This study evaluates involvement of nitric oxide (NO), angiotensin II and endothelin in renal function after ischemia/reperfusion (I/R) and identifies agents that may enhance the recovery of renal function. Renal hemodynamics and handling of water and electrolytes were determined using clearance techniques before and after ischemia induced by 20 minutes of renal artery occlusion. In ischemic kidney, GFR and RBF decreased by ~69% and ~78% respectively in the first reflow period. Urine output increased ~45% and $Na^+$ excretion increased ~13%. Without treatment, indicated parameters of kidney function slightly improved. Infusion of L-arginine and L-NAME before ischemic induction had no effect. Pretreatment with captopril (ACE-inhibitor) prevented I/R-induced changes in renal function. Similar results were observed in post-treatment with BQ-123 ($ET_A$-receptor antagonist), SNP and losartan ($AT_1$-receptor antagonist) during the reflow period. However, a slight decrease in mABP was observed in captopril, BQ-123 and SNP treatment groups but not in losartan treatment group. These results indicate changes in renal function after I/R due to lack of endogenous NO activity and increase in angiotensin and endothelin activities. This study also suggests that losartan has the most potential therapeutic use in prevention of post-ischemic decrements in renal function. (Supported by Faculty of Science, Mahidol University)

 

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***O-02***

# Renal tubular transport of the anionic chelating agent: DMPS (2-3-dimercapto-1-propanesulfonate)

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DMPS is used clinically to increase urinary excretion of heavy metals, including mercury and arsenic. We used the S2 segment of rabbit proximal tubules (RPT) to test the interaction of this anionic heavy metal chelator with basolateral transporters, Oat1 and Oat3. RT-PCR revealed expression of both transporters in single S2 segments. [$^3$H] *para*-aminohippurate ([$^3$H]PAH), and estrone sulfate ([$^3$H]ES) were used as specific substrates for rbOat1 and rtOat3, respectively. PAH and ES were transported into non-perfused single RPT segments with $K_t$s of 67 $\pm$ 20 and 3.4 $\pm$ 1.2 μM, respectively. Reduced DMPS (DMPSH) inhibited uptake of both substrates, with IC 50s of 405 $\pm$ 49 μM (for [$^3$H]PAH) and 320 $\pm$ 66 μM (for [$^3$H]ES). Oxidized DMPS (DMPSS), the prevalent from in the blood, also inhibited uptakes of [$^3$H]PAH ($IC_{50}$ of 766 $\pm$ 190 mM) and [$^3$H]ES (696 $\pm$ 166 μM). Inward gradients of ES, DMPSH and DMPSS *trans*-stimulated the 30 sec efflux of preloaded [$^3$H]ES across the basolateral membrane of RPT. Similarly, DMPSH, and PAH itseft, *trans*-stimulated the 15 sec efflux of [$^3$H]PAH. In contrast, efflux of [$^3$H]PAH was inhibited by presence of DMPSS in the bathing medium. These data suggest that, whereas both Oat1 and Oat3 transport DMPSH, DMPSS transport may be limited to Oat3. This is the fist evidence showing that both Oat1 and Oat3 can transport DMPS across the basolateral membrane of renal proximal tubules.

 

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***O-03***

# ESTROGENIC EFFECT OF WHITE KWAO KRUA (*PUERARIA MIRIFICA*) ON SERUM LIPID PROFILE AND EGG YOLK CHOLESTEROL IN LAYING HENS

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In avian species, lipid-rich yolk is synthesized in and secreted from the liver under the control of estrogen by which hepatic lipoprotein production is shifted from generic VLDL to yolk-targeted VLDL[1,4]. Supplementation of White Kwao Krua (*Pueraria mirifica*, PM), a phytoestrogen-rich herb[2], was thus tested for its potential to mediate the action of endogenously produced estrogen on serum lipid profile and egg-yolk cholesterol in laying hens.

A randomized complete block designed experiment was conducted using a total of eighty 18-week-old Newscommatt laying hens. The hens were equally divided into 4 groups receiving 4 different treatment diets for 17 weeks. The treatment diets included corn-soybean basal diet containing PM at 0, 100, 500 and 1000 ppm.

Serum lipid profile was measured enzymatically using commercial kits (Human Co., Ltd), the procedure was performed as recommended by the company. Serum estradiol was examined by Microparticle Enzyme Immunoassay using Abbott IMX$^R$. Egg yolk cholesterol was extracted by means of direct saponification[3] and quantitated enzymatically as above mentioned.

Results showed no significant differences in the hen-day egg production throughout the experimental period. At week 4 of treatment, the group fed 0 ppm PM fortified diet showed significantly higher serum triglyceride and total cholesterol levels compared to other treatment groups ($P<0.05$) while the group fed 1000 ppm PM fortified diet showed a significantly higher level of serum high density lipoprotein cholesterol level ($P<0.05$). At week 8 of treatment, a significantly higher level of serum estradiol was exhibited in the group fed 0 ppm PM fortified diet ($P<0.05$). No significant differences in egg yolk cholesterol levels were detected among the treatment groups. Discussion of the results will be presented.

1. Gruber, CJ, Tschugguel W, Schneeerger C, and Huber JC. Production and actions of estrogens. *N Engl J Med* 346: 340-352, 2002.
2. Murkies, AL, Wilcox G, and Davis SR. Phytoestrogens. *J Clin Endocrinol Metab* 83: 297-303, 1998.
3. Shen, CS, Chen IS, and Sheppard AJ. Enzymatic determination of cholesterol in egg yolk. *J Assoc Off Anal Chem* 65: 1222-1224, 1982.
4. Walzem, RL, Hansen RJ, Williams DL, and Hamilton RL. Estrogen induction of VLDLy assembly in egg-laying hens. *J Nutr* 129: 467-472, 1999.

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***O-04***

# VASORELAXATION EFFECT OF *BIOTA ORIENTALIS* EXTRACTS ON ISOLATED THORACIC AORTA OF L-NAME HYPERTENSIVE RATS

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*Biota orientalis* (BO) is one of medicinal plants that has been used in the treatment of hypertension. Our preliminary study in renovascular hypertensive rats demonstrated a reduction in blood pressure after receiving the crude extract of BO. The present study thus was to determine whether an antihypertensive effect of BO was resulted from vasorelaxation on vascular smooth muscle. Leaves from BO was dried and extracted with dichloromethane (DCM) and ethanol. Young male Sprague-Dawley rats were given L-NAME (0.5 mg/ml) in their drinking water for 3 weeks, ensuring a daily intake of L-NAME of 50 mg/kg/day (1,2). After 3 weeks, the thoracic aorta from L-NAME hypertensive rats were isolated and cut into ring segments of 3 mm. width then mounted in 15 ml organ bath filled with Krebs bicarbonate solution pH 7.4, at 37° C and continuously aerated with 95% $O_2$, 5% $CO_2$ for testing vasorelaxation effects to BO extracts. DCM fraction was the most active part of the extracts with $EC_{50}$ = 31±1.53 μg/ml, while $EC_{50}$ of ethanol fraction was 66.33±12.11 μg/ml. In addition, we studied the possible mechanisms which caused vasorelaxation of the DCM extracts. DCM extracts (66.66 μg/ml) inhibited contractile responses to angiotensin I (Ang I) with $EC_{50}$=0.07±0.04 μM in comparison with control ($EC_{50}$=0.15±0.08 μM). Furthermore, effect of DCM extract on $CA^{2+}$ evoked aortic ring contraction was investigated. DCM extract (66.66 μg/ml) inhibited $Ca^{2+}$ influx with $EC_{50}$=0.44±0.04 mM while the control was 0.37±0.05 mM. These results provide evidences of the mechanisms of vasorelaxation of BO that may confer an antihypertensive effect of the extract.

1. Hegde, L. G., P. Srivastava, R. Kumari, and M. Dikshit. Alterations in the vasoreactivity of hypertensive rat aortic rings: role of nitric oxide and superoxide radicals. *Clin Exp Hypertens* 20: 885-901., 1998.
2. Henrion, D., F. J. Dowell, B. I. Levy, and J. B. Michel. In vitro alteration of aortic vascular reactivity in hypertension induced by chronic NG-nitro-L-arginine methyl ester. *Hypertension* 28: 361-6., 1996.

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***O-05***

# HEPATOPROTECTIVE EFFECT OF *ARCANGELISIA FLAVA* MERR EXTRACTS IN MICE

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*Arcangelisia flava* is an indigenous plant that has long been used for treatment of hepatitis in several countries in the Southeast Asian including Thailand. Although it is widely used, the available information on the pharmacological actions of the plant extracts is controversy. In the present study, the chemopreventive effects of various extracts of *A. flava* against $CCl_4$-induced hepatotoxicity in mice were investigated. The plant extracts including methanol (MeOH), ethylacetate (EtOAc) and hexane (Hex) at various doses (50, 100, 250 and 500 mg/kg BW) were given 12 h prior to $CCl_4$ treatment. Administration of $CCl_4$ caused a marked elevation of several hepatotoxic markers in plasma including plasma activities of alanine transaminase (ALT) and aspartate transaminase (AST). The results showed that the methanol extract exhibited the greatest anti-hepatotoxicity against $CCl_4$. A single oral administration of the methanol extract at a dose of 250 mg/kg BW prevented the elevations of the hepatic enzymes. The possible mechanisms by which *A. flava* prevented the $CCl_4$-induced hepatotoxicity were investigated and revealed that the pretreatment of the methanol extract prevented the decreased cytochrome P-450, CYP2E1, which is the major isoform involved in $CCl_4$ bio-activation. The CYP2E1 polypeptide content, which was evaluated by Western Blot analysis, was also returned to the untreated control. *A. flava* administration also accelerated the hepatic detoxifying mechanism by increasing glutathione S-transferase activity and the amount of hepatic GSH content in $CCl_4$-treated animals. One major chemical compound in the extract was identified as berberine. It is suggested that *A. flava* exhibits an effective chemopreventive effect against the $CCl_4$-induced hepatotoxicity. *A. flava* may be of clinical value in the prevention of hepatic inflammation. However, the plant extract has a narrow margin of safety. The use of this plant must be done with caution. (Supported by Faculty of Science, Mahidol University)

Key word: *A. flava*, anti-hepatotoxic, berberine, $CCl_4$

 

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*O-06*

# STUDIES OF PROLACTIN ACTION ON SOLVENT DRAG-INDUCED ACTIVE CALCIUM TRANSPORT IN THE RAT DUODENUM

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Prolactin has recently been shown to stimulate the transcellular component of active calcium absorption. However, its action on the solvent drag-induced active calcium transport, which is the predominant component, has not been examined. The present study was performed on the duodenal segments taken from 180-200 g female Wistar rats. Ussing chamber technique was used to measure the electrical parameters and the bidirectional calcium fluxes (as calculated from the specific activity of $^{45}Ca$). The other components of calcium transport were suppressed during the course of the experiment, leaving only the solvent drag component to be measured.

Prolactin at 200, 600, 800 and 1,000 ng/ml, added directly to the serosal solution significantly ($p< 0.001$) increased the mucosa to serosa flux of calcium from a control of $11.99\pm2.07$ $nmol.hr^{-1}.cm^{-2}$ to $47.14\pm4.17$, $63.39\pm9.45$, $53.93\pm6.11$ and $29.05\pm2.78$ $nmol.hr^{-1}.cm^{-2}$, respectively. The stimulating effect of prolactin was maximum at the dose of 600 ng/ml, above which, the stimulating effect was reduced. The results confirmed the biphasic action of prolactin.

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*O-07*

# EFFECTS OF STEVIOSIDE ON GLUCOSE TRANSPORT ACTIVITY IN INSULIN-SENSITIVE AND INSULIN-RESISTANT RAT SKELETAL MUSCLE

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Stevioside (SVS) is a natural sweetener extracted from *stevia rebaudiana* and has been used for many years as an antidiabetic agent in South America. However, little is known regarding its site of action. The purpose of this study was to determine the effect of *in vivo* and *in vitro* SVS treatment on skeletal muscle glucose transport activity in both insulin-sensitive lean and insulin-resistant obese Zucker rats. SVS was administered (500 mg/kg body wt by gavage) 2 hr before an oral glucose tolerance test. Whereas the glucose incremental area under the curve ($IAUC_{glucose}$) was not affected by SVS in lean Zucker rats, the insulin incremental area under the curve ($IAUC_{insulin}$) and the glucose-insulin index (product of glucose and insulin IAUCs and inversely related to whole-body insulin sensitivity) were decreased by 27% and 32%, respectively. Interestingly, SVS reduced the $IAUC_{insulin}$ by 44%, and also significantly decreased the $IAUC_{glucose}$ (30%, $P<0.05$) and concomitantly decreased the glucose-insulin index (57%, $P<0.05$) in obese Zucker rat. Glucose transport was assessed following in vitro SVS treatment. In lean Zucker rats, basal glucose transport in type I soleus and type IIb epitrochlearis muscles was not altered by 0.01-0.1 mM SVS. In contrast, 0.1 mM SVS enhanced insulin-stimulated (2 mU/ml) glucose transport in both epitrochlearis (15%) and soleus (48%, $P<0.05$). At 0.5 mM or higher, the SVS effect was reversed. Similarly, basal glucose transport in soleus and epitrochlearis muscles in obese Zucker rats was not changed by low doses of SVS (0.01-0.1 mM). Low doses of SVS significantly increased insulin-stimulated glucose transport in both epitrochlearis and soleus (15-20%, $P<0.05$). In conclusion, SVS increased insulin sensitivity, and low concentrations of SVS (0.01-0.1 mM) improved *in vitro* insulin action on skeletal muscle glucose transport in both lean and obese Zucker rats. Further information is needed on the mechanism of SVS action following chronic administration in insulin-sensitive and insulin-resistant rodent models.

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***O-08***

# EFFECT OF CAFFEINE ON LOWER ESOPHAGEAL SPHINCTER PRESSURE IN THAI HEALTHY VOLUNTEERS

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Caffeine has been shown to affect many aspects of the body functions including the gastro-intestinal (GI) system. Its effect on upper GI functions in Thai people has never been reported.

A single-blinded study was performed to evaluate the effect of caffeine at the dose of 3.5 mg/kg on lower esophageal sphincter (LES) and esophageal peristaltic contraction in healthy Thai adults. The effect of caffeine on cardiovascular system was also studied. The volunteers were 6 men and 6 women aged 19-31 years. Each subject drank 100 ml of water. Five wet swallowing were performed 30 minutes after the drink. The basal LES pressure was continuously measured using esophageal manometric technique. They then consumed another 100 ml of water containing caffeine at the dose of 3.5 mg/kg. The basal LES pressure and swallowing were again monitored.

The experiment demonstrated that drinking 100 ml of water caused no change in basal LES pressure while caffeine consumption lowered the pressure at 10 min (13.2 $\pm$ 6.4 to 9.4 $\pm$ 5.1 mmHg, p = .036), 15 min (14.2 $\pm$ 6.8 to 9.5 $\pm$ 5.8 mmHg, p= .006), 20 min (14.6 $\pm$ 6.2 to 9.6 $\pm$ 5.5 mmHg, p= .001) and 25 min (13.9 $\pm$ 6.9 to 10.5 $\pm$ 6.7 mmHg, p= .033). The mean amplitude of contraction was also decreased at the distal esophagus (67.9 $\pm$ 46.1 to 58.4 $\pm$ 39.1 mmHg, p= .040, 112.6 $\pm$ 40.2 to 103.5 $\pm$ 42.7 mmHg, p= .038 at 3 cm and 8 cm above LES, respectively). The mean duration of contraction was decreased at the distal part (3.4 $\pm$ 0.3 to 3.1 $\pm$ 0.4 sec., p= .003) and increased at the more proximal esophagus (3.3 $\pm$ 0.6 to 3.7 $\pm$ 0.8 sec., p= .047). The mean peristaltic velocity was slightly decreased without statistical significance.

The consumption of caffeine also affected the cardiovascular system. The systolic blood pressure was increased significantly at 20 min (90 $\pm$ 9.5 to 95 $\pm$ 14.5 mmHg, p= .026). The diastolic blood pressure was increased without statistical significance (p>.05). The heart rate was increased significantly at 20-30 min after caffeine ingestion (70.7 $\pm$ 8.0 to 74.2 $\pm$ 6.7 beat/min, p= .025 and 70.7 $\pm$ 8.0 to 75.8 $\pm$ 5.1 beat/min, p= .043, respectively).

This study indicated that caffeine 3.5 mg/kg affected the esophageal function resulting in a decrease in basal LES pressure and distal esophageal contraction which promoted the reflux of gastric contents up into the esophagus. In addition, it produced an increase in blood pressure and heart rate. Individuals with upper gastrointestinal or reflux symptoms should avoid or be cautious in consuming food and beverages containing caffeine.

 

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*O-09*

# ACUTE EFFECT OF METHAMPHETAMINE ON EEG, LOCOMOTOR ACTIVITY AND STEREOTYPED BEHAVIOR IN RAT

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D-methamphetamine is the dextro-isomer of amphetamine and is one of the major components of "ya ba", the most prevalence drug among drug abuse in Thailand. The purpose of this study was to determine the acute effects of methamphetamine behaviorally. Doses of methamphetamine were administered intraperitoneally to rats and cortical electroencephalogram (EEG), locomotor activity and stereotyped behaviors were recorded. The EEGs were analyzed by quantitative power spectrum analysis. Methamphetamine was observed with a tendency to produce a decrease in power of all of the frequency bands of EEG activity. This was accompanied by a *characteristic* increase in both locomotor activity and stereotyped behaviors. This suggests that EEG power spectrum analysis with accompanying behavioral activity may prove to be a sensitive and specific investigative tool that can be used to identify those methamphetamine abuse patients.

 

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***O-10***

# *IN VITRO* HYDROPHILIC AND LIPOPHILIC ANTIOXIDANT ACTIVITIES IN PULSE EXTRACTS

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Pulses are important protein-rich foods. Twenty-three pulses were subjected to antioxidant evaluation. Four methods were employed, namely Trolox Equivalent Antioxidant Capacity (TEAC), Ferric Reducing/Antioxidant Power (FRAP), DPPH Free Radical assays. Pulses were subjected to water and ethanol extractions. Antioxidant activities of hydrophilic antioxidants were evaluated by TEAC and FRAP assays; while TEAC, FRAP and DPPH assays were used to assess lipophilic antioxidants.

FRAP detects essentially non-protein antioxidants. TEAC was found to be a sensitive tool compared to the other 2 tests as it is a rapid, convenient test. TEAC measures both protein and non-protein based antioxidants.

Results obtained from the different tests for water and ethanolic fractions were in general positively correlated. Total Antioxidant Activity (TAA) of pulses as evaluated by TEAC assay gave higher values (more than 2 times in most samples) compared to that of FRAP assay. This indicates a rich content of protein based antioxidants in pulses. The five pulses with the highest TAA were black pepper, mustard seed, peanut, red bean and rice bran. Mustard seed and black pepper are two pulses with notably strong hydrophilic and lipophilic antioxidant activities. In addition, most of the mustard antioxidant property were associated with non-protein components since the FRAP values were distinctly high.

 

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*O-11*

# EXPRESSION OF HYPOXIA INDUCIBLE FACTOR -1α mRNA IN THE NORMAL PORCINE OVARY

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Angiogenesis, mediated by vascular endothelial growth factor (VEGF), was shown to be a critical component of follicular growth and formation of corpora lutea (CL) (1, 2). Several lines of evidence showed that the production of VEGF is regulated by hypoxia inducible factor-1α (HIF-1α), especially under conditions of hypoxia (3, 4). Recently, we showed that expression of VEGF mRNA was profound in porcine non-atretic follicular and luteal tissues (5), but information on ovarian HIF-1α currently is not available. To gain insight into possible roles of HIF-1α in follicular and luteal angiogenesis, we evaluated the localization of HIF-1α in porcine ovaries that express VEGF mRNA. Northern blot analyses of total CL RNA indicated hybridization of the porcine HIF-1α probe to transcripts of approximately 3.8-kb. *In situ* hybridization showed that abundant amounts of HIF-1α mRNA are present in the follicles and CL. Within non-atretic follicles, expression of HIF-1α mRNA was intense in granulosa cells, while weaker labeling was observed in theca interna cells. These results suggest that the pig ovary provides a unique physiological model for studying HIF-1α-mediated changes in the vascular system, and HIF-1α may be a candidate regulator of ovarian VEGF expression. However, the role of HIF-1α in regulating VEGF-mediated angiogenesis in this tissue remains to be clarified.

1. Barboni, B., Turriani, M., Galeati, G., Spinaci, M., Bacci, M.L., Forni, M., and M. Mattioli. Vascular endothelial growth factor production in growing pig antral follicles. *Biol Reprod.* 63: 858-864, 2000.
2. Stouffer, R.L., Martinez-Chequer, J.C., Molskness, T.A., Xu, F., and T.M. Hazzard. Regulation and action of angiogenic factors in the primate ovary. *Arch Med Res.* 32: 567-575, 2001.
3. Blancher, C., Moore, J.W., Talks, K.L., Houlbrook, S., and A.L. Harris. Relationship of hypoxia-inducible factor (HIF)-1α and HIF-2α expression to vascular endothelial growth factor induction and hypoxia survival in human breast cancer cell lines. *Cancer Res.* 60: 7106-7113, 2000.
4. Zhong, H., De Marzo, A.M., Laughner, E., Lim, M., Hilton, D.A., Zagzag, D., Buechler, P., Isaacs. W.B., Semenza, G.L., and J.W. Simons. Overexpression of hypoxia-inducible factor 1α in common human cancers and their metastases. *Cancer Res.* 59: 5830-5835, 1999.
5. Boonyaprakob, U., Ge, Z., Hedgpeth, V.S., Routh, P., Gadsby, J.E., and G.W. Almond. In situ localization of VEGF mRNA during luteal formation and regression in the pig. *Biol Reprod.* 62, Suppl 1: Abs.no.417, p.270, 2000.

**This work was funded by the Fund for State Research Support program, NCSU College of Veterinary Medicine and all procedures for use of animals were approved by the Institutional Animal Care and Use Committee (IACUC) at North Carolina State University.**

 

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*O-12*

# EXPRESSION OF CALCIUM-SENSING RECEPTOR (CaSR) IN BOVINE MAMMARY TISSUE DURING THE PERIPARTURIENT PERIOD

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Transcellular calcium fluxes within an active mammary gland must be vigorously regulated in order to maintain mammary function and to protect calcium cytoxicity of active mammary cells. Calcium transporters, such as Plasma Membrane $Ca^{2+}$-ATPases (PMCAs), Sarco-Endoplasmic Reticulum $Ca^{2+}$-ATPases (SERCAs) and Secretory Pathway $Ca^{2+}$-ATPase (SPCA) are responsible in part for calcium transporting in the mammary gland (2, 3). However, it is not clear how calcium homeostasis in this tissue is regulated. The goal of our research is to understand the regulation of mammary gland calcium homeostasis. Extracellular calcium has been found to regulate cell functions in tissues involved and uninvolved in mineral ion homeostasis via CaSR (1). To date, a role for CaSR in mammary gland calcium homeostasis regulation has not been investigated. The objectives of this study are to determine if pregnancy and/or lactation affect the expression of CaSR in mammary gland and to investigate the relationship of the milk calcium secretion with the CaSR expressed in the mammary gland. The CaSR mRNA, quantified by using the competitive RT-PCR technique, was measured in the bovine mammary tissue collected from dairy cows at days -14, -7, 0, +7, +14, with day 0 representing calving day. The RNA/DNA ratio as an indicator of general transcription was determined. Bovine plasma calcium concentration, milk calcium secretion and its concentration were measured under the standard Atomic Absorption protocol. The RNA/DNA ratio increased almost two folds at one week prepartum and at early lactation period compared to those ratio at two week prepartum. The CaSR mRNA increased 3 times one week prepartum and remained constant through the experimental period. During periparturient period, all cows showed hypocalcemia. According to levels of hypocalcemia developed in cows, cows were grouped into severe hypocalcemic (milk fever) and mild hypocalcemic (normal) cows. The milk fever cows' total first milk calcium is higher than that of normal group. While milk fever cows secreted more calcium on the first day of lactation, the one week mammary gland CaSR mRNA expression in these cows tends to be lower than that of normal cows, though, there is not statistically different. The mammary CaSR may play neither a role in regulating whole cow calcium homeostasis nor regulating macro calcium transport in the mammary gland since there was no correlation between cows' plasma calcium and the cows' total first milk calcium to the CaRS expressed in the mammary gland. However, the CaSR expression in the lactating gland may trigger local mediator(s), such as PTHrp, within the gland, which may subsequently involve(s) in regulating calcium transport in this tissue. Further investigation should be confirmed for this hypothesis.

 

1. Brown, E. M., G. Gamba, D. Riccardi, M. Lombardi, R. Butters, O. Kifor, A. Sun, M. A. Hediger, J. Lytton, and S. C. Hebert. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature* 366: 575-80, 1993.

2. Prapong, S. Expression of bovine mammary gland $Ca^{2+}$ ATPases. In: *Ph.D Dissertation (Physiology & Biochemistry), The Graduate College*. Ames, IA 50010, USA: Iowa State University, 2000.

3. Reinhardt, T. A., and R. L. Horst. Ca2+-ATPases and their expression in the mammary gland of pregnant and lactating rats. *Am J Physiol* 276: C796-802, 1999.

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*O-13*

# EFFECT OF AGE ON LEVELS OF BLOOD CHEMISTRY PARAMETERS AND CARDIAC MARKER PROTEINS IN JUVENILE BROILER CHICKS

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The effect of age on levels of blood chemistry parameters in broiler chickens is largely unknown.[1,2] The aim of the present study was to investigate age-related physiological alterations of biochemical variables in renal, liver, lipid and cardiac profile in broiler chicks at the age of 2 (n=20) and 7 weeks (n=40). The results showed that compared with broiler chicks at the age of 7 weeks, those at the age of 2 weeks had significantly higher levels of blood urea nitrogen, creatinine, uric acid, triglycerides and the enzymes alanine aminotransferase and alkaline phosphatase. On the other hand, older chicks displayed significantly higher levels of calcium, total protein, albumin, total cholesterol and the enzyme gammaglutamyl transferase. Although a significantly higher activity of cardiac enzyme aspartate aminotransferase and a significantly lower activity of lactate dehydrogenase were observed in older chickens, younger broilers exhibited a significantly higher concentration of the cardiac-specific marker troponin T. There was no significant difference either in the levels of cardiac enzymes creatine kinase, creatine kinase MB or in glucose levels. These results imply that age is a significant contributor of variations in several blood chemistry parameters that should be considered in the interpretation of laboratory test results in broiler chicks.

1. Beker A, Vanhooser SL, and Teeter RG. Effect of oxygen level on ascites incidence and performance in broiler chicks. *Avian Dis* 39:285-291, 1995.
*2.* Maxwell MH, Robertson GW, and Moseley D. Serum troponin T concentrations in two strains of commercial broiler chickens aged one to 56 days. *Res Vet Sci* 58:244-247, 1995.

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***O-14***

# IS *Aerva lanata* (POLPALA) A DIURETIC?

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*Aerva lanata* is a medicinal plant which is used extensively in ayurvedic medicine for the treatment of renal diseases, malarial fevers, cough, lithiasis and several other disorders. It is documented in ayurvedic literature as a diuretic. Its diuretic effect was subjected to scientific screening by 2 research groups. Because of the controversial findings reported, this preliminary investigation was planned to study diuretic effect of crude aqueous plant extracts of this herb on rat animal model.

3-4 months old male Sprague-Dawley male rats weighing 200+15 g were used. Rats were fasted over night with free access to drinking water. 1 hour prior to experiment, water was given at the rate of 18 ml/kg body weight of rat. Rats were randomly divided into six groups (n=6 for each group) and each group was given one of six different solutions i.e. distilled water, 50 g/200 ml of fresh *Aerva lanata* plant extract, 100 g/200ml of fresh *Aerva lanata* plant extract, 50 g/200 ml of dried *Aerva lanata* plant extract, 100 g/200 ml of dried *Aerva lanata* plant extract and frusemide. All these solutions were administered orally at the rate of 18 ml/kg body weight of rat (This was calculated on the basis of weight ratio between the humans and the rats). Groups fed with distilled water and frusemide were considered as negative and positive controls. Animals were kept in separate metabolic cages and urine was collected for 4 hours. In order to determine the diuretic activity, urine output, urine osmolality and urinary electrolytes ($Na^+$ and $K^+$) were measured.

There was an increase in the urine output, urine osmolality and urinary $K^+$ excretion in rats treated with crude aqueous plant extract when compared with that of animals fed with water. With dried plant extract, urinary $K^+$ excretion in rats during 1$^{st}$ and 3$^{rd}$ hours was significant compared to the rats fed with water.

These data show that aqueous extracts of *Aerva lanata* have a diuretic effect.

**This research project was approved by the ethical review committee of the Faculty of Medicine, Colombo, Sri Lanka. We would like to thank Mr H Weerawarna of National Hospital of Colombo, Sri Lanka for helping us in the determination of urinary electrolytes and Mr K Perera and Mr K Gamage of Animal House, Faculty of medicine, Colombo for their assistance.**

 

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*O-15*

# DIURETIC ACTIVITY OF LOW AND HIGH MOLECULAR MASS FRACTIONS OF *Aerva lanata* (POLPALA)

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Preliminary investigations carried out by our research group found that *Aerva lanata* has a diuretic activity. In order to proceed further, investigations were carried out to determine the diuretic effect of low and high molecular mass fractions of this herb.

3-4 months old male Sprague-Dawley male rats weighing 200+15 g were used. Rats were fasted over night with free access to drinking water. 1 hour prior to experiment, water was given at the rate of 18 ml/kg body weight of rat. Rats were randomly divided into four groups (n=10 for each group) and each group was given one of four different solutions i.e. distilled water, low molecular mass fraction of *Aerva lanata*, high molecular mass fraction of *Aerva lanata* and frusemide. All these solutions were administered orally at the rate of 18 ml/kg body weight of rat (This was calculated on the basis of weight ratio between the humans and the rats). Groups fed with distilled water and frusemide were considered as negative and positive controls. Animals were kept in separate metabolic cages and urine was collected for 4 hours. In order to determine the diuretic activity, urine output, urine osmolality and urinary electrolytes ($Na^+$ and $K^+$) were measured.

There was an increase in urine output, urine osmolality and urinary electrolyte excretion in rats fed with low and high molecular mass fractions when compared with animals fed with water. This increase was significant during some hours with the low molecular mass fraction of the plant compared to groups fed with water and high molecular mass fraction.

Low molecular mass fraction of this plant has a significant diuretic activity.

**This research project was approved by the ethical review committee of the Faculty of Medicine, Colombo, Sri Lanka. We would like to thank Mr H Weerawarna of National Hospital of Colombo, Sri Lanka for helping us in the determination of urinary electrolytes and Mr K Perera and Mr K Gamage of Animal House, Faculty of medicine, Colombo for their assistance.**

 

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*O-16*

# POSSIBLE TOXICOLOGICAL EFFECTS OF *Aerva lanata* (POLPALA) ON THE URINARY TRACT OF RATS - A PRELIMINARY STUDY

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The medicinal herb, *Aerva lanata* is not prescribed for prolonged use by ayurvedic physicians in Sri Lanka as there is a common belief that it can damage the renal structure. However, there is no scientific basis for this belief. Therefore, our objective was to study any possible toxicological effects on the urinary tract of rats following long term administration of the aqueous plant extract.

3-4 months old male Sprague-Dawley male rats weighing 200+15 g were used. Rats were randomly divided into six groups (n=3 for each group) and each group received one of six different solutions i.e. distilled water, 50 g/200 ml of fresh *Aerva lanata* plant extract, 100 g/200ml of fresh *Aerva lanata* plant extract, 50 g/200 ml of dried *Aerva lanata* plant extract, 100 g/200 ml of dried *Aerva lanata* plant extract and frusemide. All these solutions were administered at the rate of 18 ml/kg body weight of rat (This was calculated on the basis of weight ratio between the humans and the rats). Groups fed with distilled water and frusemide were considered as negative and positive controls. All these solutions were given once daily for 30 days. On the 30$^{th}$ day of the experiment, animals were kept in separate metabolic cages and urine was collected for 24 hours. On the 31$^{st}$ day before the dissection, blood was obtained from their tail veins. Urine and serum creatinine levels were measured spectrophotometrically using a reagent kit to determine creatinine clearance. Kidneys, bladders and ureters of the animals were harvested through a mid ventral laparotomy under anaesthesia. These specimens were fixed in modified Boin's solution for light microscopic studies.

There was no significant difference in the creatinine clearance between groups. The histological sections of ureters and bladder from all rats did not show any structural change light microscopically. Kidneys obtained from animals treated with dried and fresh extracts of the plant showed focally congested glomeruli with no other abnormality. The kidneys from rats in other groups did not show any abnormality.

No definite histological changes were seen light microscopically in the urinary tract following administration of *Aerva lanata* plant extract.

**This research project was approved by the ethical review committee of the Faculty of Medicine, Colombo, Sri Lanka. We would like to thank Mr Muditha Kularatne, Department of Pathology, Faculty of Medicine, Colombo for processing of specimens for histological studies and Mr K Perera and Mr K Gamage of Animal House, Faculty of Medicine, Colombo for their assistance.**

 

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# THE 32nd ANNAUL ACADAMIC MEETING OF THE PHYSIOLOGICAL SOCIETY OF THAILAND

# Abstract: poster session

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***P-01***

# ANTIHYPERTENSIVE EFFECTS OF GARLIC

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Garlic has been used for its medicinal property since very old times. Enough literature is available in Indian and other textbooks about its uses. Garlic has shown antihypertensive, antiarrhythmic & cardio-protective activity along its lipid lowering effect. In this study 60 male patients aged between 35-50 yrs with high normal blood pressure and stage I of JNC Classification were included.

These patients were given raw garlic empty stomach twice daily, no other medication was used. Blood Pressure measurement was done daily and patients were followed for nine months.

Results showed that there is significant decrease in both systolic and diastolic blood pressure. This decrease is evident even after first month and was maximum at 5-6 month usage of garlic. Results also showed that this decrease in Blood Pressure was potentiated by other life style changes & dietary restrictions.

It can be concluded that along with life style changes and dietary restrictions, garlic can serve as good and effective measure to control hypertension.

 

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*P-02*

# ELECTROPHORETIC PATTERNS OF HEMOGLOBINS IN DOMESTIC FOWL

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The objective of this research was to study the hemoglobin type of domestic fowls. Blood samples from different species and ages of domestic fowls were collected to study the electrophoretic patterns of hemoglobins. Cellulose acetate electrophoretic patterns of hemoglobins of 72 adult ducks, 98 adult broiler and 80 layer hen were similar but different from 20 two weeks broiler. Hemoglobins of adult ducks and broiler showed two bands, one major (hemoglobin A, HbA) and another minor band (HbD) whereas hemoglobins of 2 weeks broiler showed 3 bands, one major, one minor and one embryonic band.

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*P-03*

# APOPTOSIS OF CIRCULATING LYMPHOCYTE IN UNILATERAL URETERAL OBSTRUCTIVE RAT : ROLE OF ANGIOTENSIN SYSTEM

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**Introduction:** During unilateral ureteral obstruction (UUO), the angiotensin system plays an important role in renal tubular cell apoptosis mediated by infiltrated lymphocytes (1, 2). No such data have revealed apoptosis of circulating lymphocyte (3, 4, 5) and the role of angiotensin system (6) in this condition. Therefore, this study aimed to examine this regard.
**Materials and methods:** The male Wistar rats were divided into two main groups: sham (S) and UUO which further treated (1 day or 7 days) with : 1) water, 2) angiotensin II receptor antagonist (ARA, losartan®: 10 mg/kg/d), and 3) angiotensin converting enzyme inhibitor (ACEI, enalapril®: 5 mg/kg/d). The levels of circulating lymphocyte, and the apoptosis of the cell (by *in situ* terminal deoxynucleotidyl transferase; TdT assay) were examined.
**Results:** The data demonstrated that UUO at both 1 day and 7 days markedly induced increases in lymphocyte apoptosis ($p<0.001$) when compared with the respective S groups. ACEI as well as ARA could reduce the heightened apoptosis in 7-day UUO rats ($p<0.001$ and $p< 0.01$, respectively), but had no effects in 1-day UUO animals. The 1-day UUO rats + ARA had a decrease in circulating lymphocyte level when compared with S ($p<0.05$) as well as with UUO + water groups ($p<0.001$). This level did not alter in 7-day UUO groups.
**Conclusion:** The present data are the first evidence that, during UUO, the angiotensin system plays a pivotal role in circulating lymphocyte apoptosis. The longer blockage of this system could abolish the induction of apoptotic lymphocyte cell. This may result in improvement of immune defense mechanism during unilateral ureteral obstruction.

1. Defranco AL and Weiss A. Lymphocyte activation and effector functions. *Curr Opin Immunol* 10: 243-267, 1998.
2. Schreiner G, Harris K, Purkerson M, and Klahr S. Immunological aspects of acute ureteral obstruction: Immune cell infiltrate in the kidney. *Kidney Int* 34: 487-493, 1998.
3. Genestier L, Bonnefony-Berade N, and Revillard J. Apoptosis of activated peripheral T cells. *Transplant Proc* 31: 33S-38S, 1999.
4. Morrissey JJ. Angiotensin II: An immune costimulator ? *Am J Kidney Dis* 36: 434-440, 2000.
5. Rudin C, Dongen J, and Thompson C. Apoptosis signalling in lymphocytes. *Curr Opin Hematol* 3: 35-40, 1996.
6. Odaka C and Mizuochi T. Angiotensin-converting enzyme inhibitor captopril prevents activation-induced apoptosis by interfering with T cell activation signals. *Clin Exp Immunol* 121: 515-522, 2000.

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***P-04***

# EFFECT OF *EUGENIA GRATA* EXTRACT ON THE IMPROVEMENT OF HEMODYNAMIC STATUS IN HEMOLYTIC ANEMIC RATS

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Hemolytic anemia is a disorder in which the red cells are prematurely destroyed. This abnormality of red cell feature found in patients with thalassemia that is the most common hereditary disease among Thai population. The disease is treated by chronic blood transfusion. However, this can cause severe iron overload resulting in progressive organ failure. It is known that phenylhydrazine (PHZ) induces oxidative damage to hemoglobin and generates free radical as well as the increase in lipid peroxidation and hemolysis. It is demonstrated that normal red cell treated with PHZ has a mimetic characteristic as those found in severe beta-thalassemia. Moreover, these PHZ-treated rats exhibited hypotension, low systemic vascular resistance and decreased vascular responses to endothelium-dependent vasodilator agents. The attenuation of vascular responses in these conditions may be due to a decrease in the effective concentration of nitric oxide (NO). Recently, our preliminary works has demonstrated that some local vegetables possess strong antioxidant activities in many assay systems. Thus, it is noteworthy to investigate the antioxidant effects of these local vegetables in this animal model of hemolytic anemia. The aim of the present study was to investigate the hemodynamic status of hemolytic anemic rats treated with *Eugenia grata* (EG), a vegetable extract. Male Sprague-Dawley rats were orally administered with EG extract (1 g/kg/day) for six days while rats in the control group were fed with deionized water. On the fourth day of treatments, animals were induced hemolysis by intraperitoneal injection of 125 mg/kg PHZ. After PHZ administration for 48 hours, animals were anesthesized and performed hemodynamic monitorings such as arterial blood pressure (MAP), hindlimb blood flow (HBF) and hindlimb vascular resistance (HVR). In the mean time, vascular responses to vasoactive substances i.e. bradykinin, acetylcholine and phenylephrine were tested. In view of hemodynamic responses, MAP and HVR of the anemic rats treated with EG were significantly increased whereas HBF was significantly decreased ($P < 0.05$). Moreover, each successive injection of vasoactive agents in anemic rats treated with vegetable EG showed progressively higher responses when compared to the control anemic rats ($P < 0.05$). Further study on the active substances that can improve the vascular endothelial function in anemic state remains to be explored.

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*P-05*

# ROLE OF ANGIOTENSIN SYSTEM IN UNILATERAL URETERAL OBSTRUCTION : RENAL eNOS PROTEIN EXPRESSION, NEPHROPATHY, AND URINARY ELECTROLYTE LEVELS

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**Introduction:** Unilateral ureteral obstruction (UUO) could cause alterations in renal hemodynamics and structures (1). The angiotensin system (2) as well as nitric oxide (3, 4)play critical roles during UUO. However, no clear studies have demonstrated the role of angiotensin system on renal eNOS protein expression in this condition. Thus, we aimed to examine this regard.
**Materials and methods:** The male Wistar rats were divided into two main groups: sham (S) and UUO. In UUO group, the animals were further divided into 3 subgroups treated with: 1) water, 2) angiotensin II receptor antagonist (ARA, losartan®: 10 mg/kg/d), and 3) angiotensin converting enzyme inhibitor (ACEI, enalapril®: 5 mg/kg/d) for 7 days. The renal eNOS expression (by immunohistochemistry) and renal tissue damage (by PAS and Masson's technique) were examined. The urinary concentrations (U) of Na, K, and Cl in bladder and pelvis also were determined.
**Results:** The data showed that UUO increased eNOS expression mainly in medulla, and caused renal tissue damage about 50-75% (grade 3) when compared with S animals. Both ACEI and ARA could further enhance eNOS expression (both in cortex and medulla); however, only ACEI could diminish renal tissue damage. The UUO animals had the lower U Na, K, and Cl in pelvis than those in bladder ($p<0.001$). ACEI and ARA could restore only U Na and K back to comparable levels as in bladder.
**Conclusion:** The present data show the first evidence that the angiotensin system plays a critical role in modulation of renal eNOS protein expression, nephropathy, as well as urinary electrolyte concentration during UUO.

1. Klahr, S. Urinary tract obstruction. *Semin Nephrol* 21: 133-145, 2001.
2. Pimentel JL, Martinez-Maldonado M, Wilcox JN, Wang S, and Luo C. Regulation of renin-angiotensin system in unilateral ureteral obstruction. *Kidney Int* 44: 390-400, 1993.
3. Hegarty NJ, Young LS, Kirwan CN, O'Neill AJ, Bouchier-Hayes DM, and et al. Nitric oxide in unilateral ureteral obstruction: Effect of regional blood flow. *Kidney Int* 59: 1059-1065, 2001.
4. Huang A, Palmer LS, Hom D Valderrama E, and Trachman H. The role of nitric oxide in obstructive nephropathy. *J Urol* 163: 1276-1281, 2000.

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*P-06*

# ANGIOTENSIN SYSTEM REGULATES RENAL eNOS PROTEIN EXPRESSION, NITRIC OXIDE PRODUCTION, AND ELECTROLYTE EXCRETION DURING RENAL ISCHEMIC REPERFUSION

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**Introduction:** Renal ischemic reperfusion (IR) could lead to end organ failure (1, 2). Nitric oxide (NO) plays an important role to maintain the perfusion (3, 4, 5). However, no studies have investigated the renal eNOS protein expression and the role of angiotensin system (6) in this condition. Thus, we aimed to examine this regard.
**Materials and methods:** The male Wistar rats were divided into two main groups: sham (S) and IR (for 30 min.). In IR group, the animals were further divided into 3 subgroups treated with: 1) water, 2) angiotensin II receptor antagonist (ARA, losartan®: 10 mg/kg/d), and 3) angiotensin converting enzyme inhibitor (ACEI, enalapril®: 5 mg/kg/d) for 1 day. Serum NO levels (by ELISA), and eNOS expression (by immunohistochemistry) were examined. Na and K excretion also were determined.
**Results:** The data demonstrated that IR increased NO production ($p<0.05$) whereas the eNOS expression showed less staining ($p<0.05$) when compared with S animals. Fractional excretion (FE) of Na ($p<0.01$) and of K ($p<0.05$) were diminished. Both ACEI and ARA could attenuate the heightened NO levels ($p<0.01$ and $p<0.001$, respectively). However, only ACEI could restore the eNOS expression while only ARA could rebound $FE_{Na}$ and $FE_K$ to the same levels of S group.
**Conclusion:** The present data show the first evidence that the angiotensin system plays a crucial role in regulation of renal eNOS protein expression, NO production, as well as electrolyte excretion during renal ischemic reperfusion.

1. Lerman L and Textor SC. Pathophysiology of ischemic nephropathy. *Urologic Clinics of North America* 28: 793-803, 2001.
2. Sheridan AM and Bonventre JV. Cell biology and molecules mechanisms of injury in ischemic acute renal failure. *Curr Opin Nephrol Hypertens* 9: 427-434, 2000.
3. Goligorsky MS and Noiri E. Duality of nitric oxide in acute renal injury. *Semin Nephrol* 19: 263-277, 1999.
4. Saito, M., and I. Miyagawa. Real time monitoring of nitric oxide in ischemia-reperfusion rat kidney. *Urol Res* 28: 141-146, 2000.
5. Waz WR,Van JB, and Feld LG. Nitric oxide metabolism following unilateral renal ischemia/ reperfusion injury in rats. *Pediatr Nephrol* 12: 26-29, 1998.
6. Zbou MS, Adam A, and Raij L. Interaction among angiotensin II, nitric oxide and oxidative stress. *JRAAS* 2: S59-S63, 2001.

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*P-07*

# BLUNTED RENAL PRESSURE-DIURESIS/NATRIURESIS IN ADULT CONSCIOUS RATS SUPPLEMENTED BY TAURINE IN THE EARLY LIFE

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Taurine plays important roles in cell growth and differentiation. Previous experiments indicate that depletion of taurine decreases renal function in adult animals. In contrast, its supplementation improves renal function in rats with nephrotic syndrome. This study tests the hypothesis that either depletion or supplementation of taurine in the early life alters renal function in adult conscious rats. Female Sprague Dawley (SD) rats were fed with a normal rat chow and tap water containing 3% β-alanine (taurine depletion), 3% taurine (taurine supplementation) or water alone (Control) either from conception until delivery (fetal period) or from delivery until weaning (lactation period). After weaning, male offspring's were fed with the normal rat chow and tap water *ad libitum*. At 7-8 weeks of age, all rats were implanted with femoral arterial, venous and bladder catheters. Forty-eight hours later, arterial pressure and heart rate were continuously recorded in a conscious restrained condition. Urine and blood samples were collected before, during, and after intravenous saline infusion (5% body weight, 0.5 ml/min). Body weight, kidney weight, and kidney weight to body weight ratio were not significantly different among groups, whereas heart weight of the taurine depleted fetus group (TDF) was slightly higher than other groups. Mean arterial pressures were higher in rats supplemented with taurine in either fetal (TSF group) or lactation period (TSL group) when compared to Control, TDF or taurine depletion during lactation (TDL group) (baseline: Control 111 ± 2 mm Hg, TDF 113 ± 2 mm Hg, TDL 116 ± 1 mm Hg, TSF 120 ± 3 mm Hg, TSL 120± 2 mm Hg; $P < 0.05$), while heart rates of all treated groups were not significantly different from the control. Basal plasma sodium concentrations were not significantly different among groups, whereas basal plasma potassium of the TSF group was significantly ($P < 0.05$) lower than the others (Control 4.0 ± 0.1 mEq/L, TDF 3.4 ± 0.2 mEq/L, TDL 4.2 ± 0.3 mEq/L, TSF 3.3 ± 0.2 mEq/L, TSL 3.6 ± 0.1 mEq/L). Despite higher mean arterial pressure, adult rats with taurine supplementation in the fetal and lactation periods displayed diuretic and natriuretic responses to an acute saline load similarly to other groups (percent water excreted in 90 minutes per gram kidney weight: Control 44.7 ± 6.7 %, TDF 38.8 ± 5.8 %, TDL 36.4 ± 6.1 %, TSF 41.2 ± 4.7 %, TSL 43.5 ± 3.7 % ; percent sodium excreted in 90 minutes per gram kidney weight: Control 44.7 ± 6.0 %, TDF 37.8 ± 4.4 %, TDL 44.1 ± 7.0 %, TSF 38.9 ± 4.4 %, TSL 42.2 ± 3.0 %). The present data suggest that taurine supplementation in the early life elevates arterial pressure and blunts pressure-diuresis/natriuresis in adult conscious rats.

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***P-08***

# EFFECT OF HISTAMINE ON MESENTERIC MICROCIRULATION, MEASURED BY INTRAVITAL MICROSCOPY

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Using in vivo intravital microscopy, we examined the role of Histamine on mesenteric microcirculation. The present experiments were carried out on 25 male Sprague –Dawley rats anaesthetized by subcutaneous administration of 1.25ml of 20% solution of Urethane per 100gram body weight of animal (1) and prepared for in vivo intravital microscopy of mesenteric microcirculation. The internal diameter (i.d) of the Terminal arterioles (T°A) as measured through a precalibrated micrometer eye-piece fitted to the binocular microscope (Hertell and Reuss) was found to be 18-23µm(microns), these T°A branch out to the capillaries of 4-9µm.

One mMol solution of Histamine in modified tyrode solution pH- 7.4, was topically applied on the preparation and the i.d. of the vessels was measured to know the latency and magnitude of the response, any change in i.d. of the vessels was expressed as the percentage of control, the vascular flow was visually recorded. Soon after application (10-20 sec) the arterioles were found to be in dilated state, and the i.d. became almost twice the control diameter between 80-100 sec. This gradually approached the control diameter in 5 minutes. In most instances the number of dormant capillaries increased by 50-60%, the flow rate in the active capillaries also substantially increased.

The effect presumably appears to be either a direct effect on Histamine receptors or on the specific ion-channels, thus bringing about the relaxation of the smooth muscles of the terminal arterioles. It seems that the local regulatory mechanisms like the hormones and transmitters are of greater value than the enteric neural regulation alone. The possibility that the specific histaminergic receptors are present on intestinal microcirculation has not been explored in the current work, as it requires rather more comprehensive experimental work.

1. Marshall JM and Tandon HC.Functional hyperaemia in muscle micro vessels. J Physiol (London) 350:447-459, 1984.

 

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***P-09***

# CHRONIC EXPOSURE OF CULTURE RAT HIPPOCAMPAL NEURONS TO SUB-LETHAL DOSE OF SYNTHETIC BETA AMYLOID PEPTIDE (β25-35) ENHANCES NEUROTOXICITY OF EXOGENOUS NITRIC OXIDE GENERATOR

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β-Amyloid is the major protein component of neuritic plaques enormously found in the brain of Alzheimer's Disease patients. The studies showed that the peptide has *in vitro* neurotoxic effects (3). Nevertheless, tentative neurodegeneration mechanism of the peptide is obscure. The fact that reactive glial cells commonly localized near neuritic plaques may indicate their possible concern with neurodegenerative processes in that region. The in vitro studies showed that β-Amyloid fragment (β25-35) with specific cytokines or β-Amyloid peptide (β1-40) alone can stimulate glial cells to generate nitric oxide more than usual (1, 2). The relationship of β-Amyloid and nitric oxide in causing neurodegeneration has not been determined. In our study we cultured hippocampal neurons from day14-rat fetus on 24 well-plates with or without sub-lethal dose (10 μM) synthetic β-Amyloid (β25-35) coating. After day4 of post plating, the culture was treated with 1 μM SNP (Sodium nitroprusside), an exogenous nitric oxide generator. Culture media was collected for 3 consecutive days to measure LDH activity. We found that exposing culture rat hippocampal neurons to sub-lethal dose of synthetic β-Amyloid peptide (β25-35) for 4 days of post plating induced early and severe degeneration to the neurons when post exposed to 1 μM SNP. Our result suggested that chronic exposing culture neurons to sub-lethal dose of β-Amyloid appeared to be more susceptible to toxicity of nitric oxide from SNP than the control culture.

1. Goodwin JL, Uemura E, and Cunnick JE. Microglial release of nitric oxide by the synergistic action of β-amyloid and IFN-γ. *Brain Research* 692: 207-214, 1995.
2. GoodwinJL, Kehrli Jr. ME, and Uemura E. Integrin Mac-1 and β-amyloid in microglial release of nitric oxide. *Brain Research* 768: 279-286, 1997.
3. Selkoe DJ. The molecular pathology of Alzheimer's disease. *Neuron* 6: 487-498, 1991.

 

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***P-10***

# *P53* CODON 72 POLYMORPHISM AND SELECTED RISK FACTORS IN CERVICAL CANCER PATIENTS AT SRINAGARIND HOSPITAL

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*p*53 is a tumor-suppressor gene which located at a short arm of chromosome 17. The gene functions include regulation of the cell cycle and the cellular response to DNA damage, initiation of DNA repair and replication, and induction of apoptosis as well as promotion of cell differentiation. It has been reported that the polymorphism of this tumor suppressor gene especially at codon 72 may be associated with an increased risk of several cancers. Whether subjects carrying the arginine form of *p53* gene are more susceptible to cervical cancer than those carrying the proline form remains controversial. Therefore, the present study was aimed to elucidate the role of *p*53 codon 72 polymorphism in cervical carcinoma at Srinagarind hospital. The genetic polymorphism of this tumor suppressor gene was determined in genomic-DNA of squamous cell cervical carcinoma patients (n=41) and controls (n=47) by PCR-RFLP. It was indicative that there was no statistical difference in cervical carcinoma risk between the two groups. In addition, the results obtained from interviewing of 88 cervical carcinoma patients and 100 healthy control subjects revealed that the number of sexual partners and age at the first sexual intercourse are the risk factors for cervical carcinoma.

 

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*P-11*

# OXYGEN RADICAL SCAVENGING ACTIVITY OF COMMON VEGETABLES IN ACTIVATED MACROPHAGES

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Vegetables are the favorite Thai food. Thai people enjoy a variety of local vegetables in daily diet. Northeast region of Thailand is one of a rich source of vegetables. Ecological and epidemiological evidences suggest that large amount of consumption of fruit and vegetables is associated with reduced risk of various diseases i.e. cancer, inflammation and cardiovascular diseases. Several mechanisms have been proposed for protection, including an increased nutrient intake of antioxidant compounds. The present study was thus designed to determine whether these local vegetables possess the antioxidant propoties in inhibit of $H_2O_2$ production in activated macrophages. Eight common vegetables were collected and one portion was freshly extracted with water and another was dried before being extracted with ethanol. Antioxidant activity was assessed by the inhibition of $H_2O_2$ production in activated rat peritoneal macrophage by using fluorescent dichlorofluorescein probe. Parameters, $E_{max}$ and $E_{50}$ values, were derived from the efficiency of the common vegetable extracts in inhibiting the $H_2O_2$ generation in activated macrophages. $EC_{50}$ values of vegetable extracts, e.g. Neam-hoo-sua, Bai Chaplu and Paak Kadheen, were 6±0.6, 28±5.5 and 109±5.9 μg/ml, respectively. Moreover, it was appeared that ethanol extracts of these vegetables were potent than water extracts. The results indicated that local vegetable extracts possess antioxidant property by oxygen radical scavenging activity. They may thus be beneficial for the prevention of various diseases.

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***P-12***

# ANTIDEPRESSANT AND ANXIOLYTIC EFFECTS OF SELECTED UREA ANALOGUES OF CCK-ANTAGONISTS

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In this study, two novel synthetic urea analogues previously showed to be cholecystokinin (CCK) antagonists, especially at $CCK_B$ receptor, were studied for their CNS effects. Mice were used and divided into 5 groups (n=8 in each group). Animals were injected intraperitoneally with either 50% propylene glycol as control, N- (1,5-dimethyl-3-oxo-2diphenyl-2, 3-dihydro-1H-pyrazol-4-yl)-N-(1-napthyl) urea (1 or 5 mg/kg) or N- (5 dimethyl-3-oxo-2diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(1H-indol-3-yl)propanamide (1 or 5 mg/kg). Animals were assessed for their behavioural changes at 30 minutes after treatment. The analgesic effect was evaluated by using the tail flick and the hot plate tests, the anxiolytic effect by the elevated X-maze and the light and dark box tests and the antidepressant effect by the tail suspension and the forced swim tests. In addition, muscle power of the rodents was also measured by wire mesh and the rota-rod tests. The results showed that, N- (5 dimethyl-3-oxo-2diphenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(1H-indol-3-yl) propanamide at a dose of 5 mg/kg had an antidepressant and an anxiolytic effect in the forced swim and the light and dark box tests, respectively. Whereas N- (1,5-dimethyl-3-oxo-2diphenyl-2, 3-dihydro-1H-pyrazol-4-yl)-N- (1-napthyl) urea (1mg/kg) showed an anxiolytic effect only in the tail suspension test. Both of substances selected were not displayed the analgesic effect or the effect on muscle power of rodents.

 

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***P-13***

# GLUTAMATE TREATMENT ALLEVIATES NEUROPATHY IN PYRIDOXINE- BUT NOT IN ACRYLAMIDE-INDUCED TOXICITY IN RATS

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Glutamate (Glu), one of the excitatory neurotoxin, has been reported to have neuroprotective effects against many cytotoxic agents, based on different mechanisms (3,4). Pyridoxine (B6) megadose (2) and acrylamide (Acr) (1) are neurotoxic agents and have been reported to be the causes of neuropathy found in both human and animals. The aim of this study was to investigate the neuroprotective effects of Glu in B6- and Acr-induced toxicity in rats. Sprague Dawley rats were divided into 6 groups and the treatment period was 14 days. Group 1 served as control. Group 2 received only Glu (500 mg/kg/day) orally. Group 3 were IP injected with B6 (800 mg/kg/day) Group 4 were IP injected with B6 (800 mg/kg/day) in combination with Glu. Group 5 was IP injected with Acr (50 mg/kg q 48 h). Group 6 received Glu orally in combination with Acr IP injection. Feeding of Glu was started 1 day prior to the IP injection. The animal motor coordination, the muscle power, and thermal threshold were observed daily. The nerve conduction velocity was measured on day 0 and day 15. Glu alone appeared to have no effects on all parameters compared to those of the control. In B6-induced toxicity rats, Glu alleviated the toxic effects of pyridoxine in all parameters determining for peripheral neuropathy. In contrast, Glu worsen the toxic effects.of Acr in Acr-induced neurotoxic rats.

1. Al Deeb S., Al Moutaery K., Arshaduddin M., Biary N., and Tariq M. Attenuation of acrylamide-induced neurotoxicity in diabetic rats. *Neurotoxicol Teratol.* 22:247-253, 2000.
2. Berger A.R., Schaumburg H.H., Schroeder C., Apfel S., and Reynolds R. Dose response, coasting, and differential fiber vulnerability in human toxic neuropathy: a prospective study of pyridoxine neurotoxicity. *Neurology.* 42: 1367-1370, 1992.
3. Boyle F.M., Wheeler H.R., and Shenfield G.M. Glutamate ameliorates experimental vincristine neuropathy. *J Pharmacol Exp Ther*. 279: 410-415, 1996.
4. Boyle F.M., Wheeler H.R., and Shenfield G.M. Amelioration of experimental cisplatin and paclitaxel neuropathy with glutamate. *J Neurooncol.* 41 107-116, 1999.

 

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***P-14***

# THE EFFECT OF SUBSTITUTION OF CASSAVA FOR CORN IN BROILER DIETS ON GASTROINTESTINAL GROWTH AND FUNCTIONS

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Differences in physical and chemical properties of feedstuffs that influence in vivo digestibility and fermentability may influence gastrointestinal growth and functions. This experiment was to investigate such effect as a result of substitution of cassava for corn in broiler chickens. Twenty-four one-day old Cobb broiler chicks were divided into 3 groups of 8 animals each, and kept individually in metal cages where feed and water were provided ad libitum. The chicks in group I, II, and III were randomly fed semipurified diets containing corn, cassava chips and cassava pellets, respectively, until 28 days of age. The diets were isonitrogenous and isocaloric and fed in pelleted form. At the end of the experiment, the chickens were euthanized and proventriculus, gizzard, small intestine (duodenum, jejunum and ileum), caeca, pancreas, liver and spleen were weighted and the intestinal length were measured. Sucrase and maltase activity in duodenum and sucrase activity in jejunum of the broilers fed corn containing diet were significantly lower ($P<0.05$) than those fed cassava chip and cassava pellet containing diets. There were no significant differences in mucosal protein and sucrase and maltase activities in ileum of broilers in all 3 groups. Broilers fed cassava chips showed lower ($P<0.05$) gizzard weight, higher liver weight ($P<0.05$), and higher percentage of caecal butyrate than those fed corn or cassava pellet diets.

1. Bird, AR, Brown IL, and Topping DL. Starch, resistant starches, the gut microflora and human health. *Curr Issues Intest Microbiol* 1 (1): 25-37, 2000.
2. David, L, and Peter M. Short-chain fatty acids and human colonic function : roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews* 81 (3): 1031-1066, 2001.
3. Jacobasch, G, Schmiedl D, Kruschewski M, and Schmehl K. Dietary resistant starch and chronic inflammatory bowel diseases. *Int J Colorectal Dis* 14: 201- 211, 1999.
4. McAllister, TA, Philippe RC, Rode LM, and Cheng, KJ. Effect of the protein matrix on the digestion of cereal grains by ruminal microorganism. *J Anim Sci* 71: 205-212, 1993.
5. Weurding, RE, Veldman A, Veen WAG, and Van der Aar PJ. Starch digestion rates in the small intestine of broiler chickens differs among feedstuffs. *J Nut* 131: 2329-2335, 2001.

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