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cAMP SIGNAL TRANSDUCTION IN THE LIVER OF PROTEIN-ENERGY MALNOURISHED (PEM) RATS. L.L. Stephen and L.E. Nagy, Dept. Nutritional Sciences, University of Guelph, Guelph, Ontario, N1G 2W1

Changes in hormonal levels during PEM have been well characterised. However, cellular responses to hormonal stimulation during PEM are not well understood. Recent evidence indicates that cAMP mediated responses are desensitized in liver during PEM. The objective of this study was to investigate the mechanism for this desensitization. Fifty six (56) weanling rats were fed either a 0.5% or 15% protein diet for 1, 3, 7 or 14 days. Hormone stimulated adenylyl cyclase activity was increased in hepatocyte membranes of PEM rats compared to controls at day 14. This may be due in part to changes in the guanine nucleotide regulatory protein, as the quantity of the α_s subunit increased by 1.8 fold by day 14 of PEM, whereas the α_i subunit was not changed. Despite increased cAMP production in PEM rats, protein kinase A (PKA) activity was decreased to 1571 ± 309 pmol/min/mg protein in liver cytosol of PEM rats compared to 3135 ± 766 in control rats ($p<0.05$) by day 3. This decrease persisted to days 7 and 14 of PEM. These data indicate that increases in cAMP in livers of PEM rats are due, in part, to an increase in α_s at day 14. However, the rapid decrease in PKA activity indicates that desensitization of cAMP signal transduction occurs at early stages of PEM.

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REGULATION OF CHOLESTEROL 7α-HYDROXYLASE BY FEMALE SEX STEROIDS. M-C. Qiu, J. Gilloteaux, T.R. Kelly and J.Y.L. Chiang. Depts. of Anatomy and Molecular Pathology and Biochemistry, NEOU College of Medicine, Rootstown OH 44272.

Cholesterol 7α-hydroxylase (P450c7) mRNA level and activity can be regulated by the female sex hormones estradiol (E) and medroxyprogesterone (MP), under different dietary conditions, i.e., cholesterol (CH) and cholestyramine (CA). Female Syrian hamsters were kept at 20°C, water ad lib, and submitted to reverse day/night cycles, to one-month treatments (n=3 to 6), and weekly treated. Seven treatments were studied: Control (C; .5 ml corn oil alone), E (benzoate, 5 μg/100 gm. b.w. in oil, i.p.), MP (MP acetate, 7-8 mg/100 gm b.w.i.m.), and E+MP combination (same doses as in E+MP), CH-fed (1%), and CA-fed (5%). Hepatic structural changes were observed; microsomes and mRNA were extracted and purified from each hamster. The expressions of liver P450c7 mRNA activity were decreased by MP and by E+MP ($p<.05$). However, no significant change in mRNA level and P450c7 activity were detected after E treatment alone. Dietary cholesterol significantly induces P450c7 activity ($p<.05$). In contrast, P450c7 mRNA levels were not increased significantly. From these preliminary findings, it is suggested that the cholesterol feeding might feedforward regulate P450c7 in the hamster. Sponsored by Summa Health System Foundation, Akron and NIH grant GM31584.

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A DECREMENT IN GLYCEMIA OF SHORT DURATION CONFOUNDS THE COUNTERREGULATORY RESPONSE DURING HYPOGLYCEMIC CLAMPS. M. Hamilton-Wessler, R.N. Bergman, J.B. Halter, and C.M. Donovan, Depts. of Exercise Science and Physiology & Biophysics, University of Southern California, Los Angeles, CA 90033; Institute of Gerontology, University of Michigan, Ann Arbor, MI 48109.

Hypoglycemic glucose clamps have been utilized in the study of hormonal mechanisms in glucose counterregulation which may be deficient in IDDM (Bolli *et al*, 1984). However, the initiation of insulin infusion may result in a transient hypoglycemic "dip" which may impact upon the counterregulatory response during the early phase of the hypoglycemic clamp. We examined the influence of a short-term dip in glycemia upon the subsequent counterregulatory response in chronically cannulated dogs (N=5). General systemic hypoglycemia was induced via an insulin infusion of 6.5 mU/min/kg for a period of 150 minutes. Glucose was infused via the portal vein to establish an arterial glycemia of 47±2 mg/dl during minutes 30-150 of the experimental period. In *Group 1*, the hepatic glycemia was allowed to fall to a nadir of 54±2 mg/dl at 20 minutes (P=0.0017 vs. Group 2) and was then restored to 74±6 mg/dl by minute 30, with maintainenance at 70±1 mg/dl over the final 120 minutes. In *Group 2*, the initial glycemia was controlled so as to clamp hepatic glycemia at 67±3 mg/dl by minute 20 and then maintain it at 70±2 mg/dl throughout the remaining experimental period. Early phase glucagon (GLG) and epinephrine (EPI) responses above basal were demonstrated to be greater in Group 1 when compared to Group 2 (ΔGLO of 218±11 vs. 64±26 pg/ml at minute 30, P=0.0016; and ΔEpi of 1537±278 vs. 623±125 pg/ml at minute 60, P=0.015). No differences between groups were noted in the norepinephrine responses. These data demonstrate that a transient fall in glycemia at the beginning of a clamp will by itself generate a counterregulatory response and it must be prevented when examining the counterregulatory response to insulin-induced hypoglycemic clamps. Supported by JDF grant 191307, and NIH grant DK27619

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OVULATORY RESPONSES OF ADULT MICE TREATED NEONATALLY WITH ESTRADIOL OR METHOXYCHLOR (TECHNICAL GRADE). V.P. Eroschenko, W. J. Swartz, and L. C. Ford, Dept. of Biological Sciences, University of Idaho, Moscow, ID 83844 and Dept. of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

Methoxychlor (MXC), a widely used organochlorine pesticide is estrogenic. To determine its effects on ovulation, one-day-old mice were exposed daily for 14 days to either sesame oil or 10.0 μg estradiol-17 β (E), or 0.1 mg, 0.5 mg, or 1.0 mg MXC suspended in sesame oil. At two and four months of age, the animals were injected with a superovulatory regimen of 10 I.U. of pregnant mare's serum followed by 10 I.U. of human chorionic gonadotropin. The chemical treatments produced both a time- and dose-dependent changes. Ovulatory responses and ovarian weights were significantly reduced by two months of age in mice neonatally exposed to E, 0.5 mg, or 1.0 mg MXC. By four months of age, all treated animals exhibited a significant reduction in number of oocytes ovulated in response to exogenous gonadotropins. The ovaries in the treated mice also exhibited fewer fresh corpora lutea and an increase in the number of atretic follicles. It is concluded that neonatal methoxychlor exposures alter ovarian responses to exogenous gonadotropins, resulting in decreased ovulations. Supported in part by NIOSH grant OH00835

THERMOGENESIS AND BROWN ADIPOSE TISSUE (5556-5557)

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ALTERATIONS IN COLD-EXPOSED THERMOGENIC CAPACITY OF SENESCENT F344 RATS. M.L. Florez-Duquet, A.M. Gabaldon, J.S. Hamilton, B.A. Horwitz, R.B. McDonald, Dept. of Nutrition and Section of Neurobiology, Physiology, and Behavior, DBS, Univ. California, Davis, CA. 95616

Previous investigations have indicated that body temperature of cold-exposed 26 month-old male F344 rats decreases to a greater degree than does that of age-matched females. This age/gender difference may reflect alterations in the thermogenic contribution of brown adipose tissue (BAT) non-shivering thermogenesis and/or skeletal muscle shivering thermogenesis. To test this possibility, we measured indices of BAT and skeletal muscle thermogenic capacity in warm-(25C) and cold-exposed (6C) male and female F344 rats, ages 6, 12, and 26 months. As expected, core temperature of the cold-exposed 26 month-old male versus female rats was significantly lower. Although skeletal muscle glycogen levels decreased during cold exposure in all groups, glycogen utilization and oxidative capacity did not differ with age or gender. In contrast, BAT mass and the expression of uncoupling protein in 26 month-old male rats was significantly less than that observed in female rats. These, as well as previously reported data, strongly suggest that the gender related differences in the ability to maintain homeothermy in the cold-exposed senescent rat are more likely to reflect alterations in BAT quantity and quality rather than differences in skeletal muscle thermogenic capacity. (Supported by NIH AG06665, AG05577, and GM159229.)

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A Possible Mechanism of Uncoupling Action of Anacardic Acid (AA) on Oxidative Phosphorylation: simultaneous determination of membrane potential (ΔΨ) and transmembrane pH difference (ΔpH) in liposomes. M.Toyomizu, K.Okamoto, T.Nakatsu, T.Konishi (SPON.:M.T.Clandinin). Animal Nutrition, Tohoku Univ. & Niigata Univ., Japan; Takasago Inst., USA; Radiochemistry-biology, Niigata Coll. of Pharmacy, Japan

We have previously shown that AA has the uncoupling action on oxidative phosphorylation in rat liver mitochondria. The present studies were undertaken to clarify whether the AA acts as a protonophore or an ionophore. Large unilamellar liposomes were prepared by the reverse-phase evaporation method. Both changes of ΔΨ and ΔpH were determined by photodiode array spectrometry, using a cyanine dye (diS-C_3(5)) and 9-aminoacridine (9-AA) as the probes, respectively. AA (f.c. 500nM) quenched the diSC_3 fluorescence but the extent was far less than that by Val, nor did subsequent addition of FCCP form ΔpH at all, implying that AA has little K^+-ionophore activity at this concentration. The ΔΨ formed by Val-K^+ was not affected at AA concentration lower than 300 nM whereas it decreased gradually in the range of 300-5000nM. AA partly dissipated K^+-diffusion potential formed by Val added previously, but ΔΨ driven, AA-mediated H^+-influx process was not observed, indicating that AA does not act as a protonophore. The initial rate of the AA-mediated ΔΨ dissipation were exponentially correlated to the logarithmic concentration of AA and it also depended on the magnitude of ΔΨ formed by Val-K^+. These results suggested that lipid-soluble AA^- may act as (-)-charge carrier.

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