INTERACTION OF NITROUS OXIDE AND METHOTREXATE. Michael J. Combs, Thomas Guse, and Robert F. Schilling, Department of Medicine, University of Wisconsin, Madison, WI

Nitrous oxide, (N_2O) so commonly used as an analgesic and anesthetic agent, inactivates vitamin B_{12} dependent enzymes in vivo and in vitro. Because of the intimate biochemical inter-dependency of vitamin B_{12} and folate we tested rats for an interaction between N_2O and the anti-folate, methotrexate. Groups of 10 weanling rats, raised on vitamin B_{12} sufficient or deficient diets, were exposed or not exposed to N_2O, and then given methotrexate by injection. The end point used was time to death. Rats living at 10 days (the end of our experiment) were recorded as having survived 10 days. Data were analyzed by the log-rank test statistic. Methotrexate is significantly more lethal in animals exposed repeatedly to N_2O, and in B_{12} deficient rats a single 3 hour exposure to 50% N_2O resulted in significantly enhanced mortality from methotrexate. In B_{12} sufficient rats a single exposure to N_2O did not increase the mortality after methotrexate. These data are consistent with other published information suggesting the N_2O is likely to cause significantly increased toxicity from methotrexate. Studies to test the efficacy of leucovorin rescue after N_2O exposure are needed.

ENDOCRINOLOGY

DIFFERENT PRL AND GH RESPONSES TO VASOACTIVE INTESTINAL PEPTIDE IN PATIENTS WI HYPERPROLACTINEMIA. MA Arnaout,* R Amari,* KM Ajlouni,* DR Martinson,* TL Garthwaite TC Hagen. Department of Medicine, VA Medical Center, Medical College of Wisconsin, Milwaukee WI and Faculty of Medicine, University of Jordan, Amman, Jordan.

VIP is a putative hypothalamic PRL releasing factor. VIP administered IV stimulates the relea of PRL in normal subjects but not in patients with prolactinoma. Since VIP stimulates greater PR release *in vitro* from micro- than macroadenomas, we studied the PRL, LH, FSH, and GH responses VIP in patients with either microadenomas or idiopathic hyperprolactinemia (MI) versus patients wi macroadenomas (MA).

Eleven patients with current or previous hyperprolactinemia were studied. In patients who had be receiving bromocriptine therapy, the therapy was discontinued for ≥6 weeks prior to study. M consisted of 4 female patients, aged 21-27 yr, 2 of whom had no demonstrable tumor on CT scan, a 2 of whom had therapeutic resolution of microadenomas, with normal prolactin levels currently. M consisted of 7 patients (2 males, 5 females), aged 21-44 yr. Following a 30 minute equilibrati period, synthetic porcine VIP in normal saline was given as a single IV bolus dose of 1 μg/kg. Bloo samples were obtained at intervals after VIP injection. On a second day, the protocol was repeated wi GnRH (100μg) and TRH (500μg) in place of VIP.

After VIP injection, plasma VIP levels increased significantly at 5, 15 and 30 min. PRL increase ($p<0.05$) over basal in MI, but not in MA patients. The net PRL release (peak minus basal) w significantly ($p<0.005$) higher in MI than in MA patients. TRH stimulated PRL release in bo groups, and the net increase was significantly greater ($p<0.025$) in MI than MA patients. Similar VIP stimulated the release of GH in both groups, and the net increase in GH release was great ($p<0.025$) in MI than in MA patients (increment of 6.8 ± 1.4 versus 2.8 ± 0.7 ng/ml respective [M±SEM]). VIP did not stimulate the release of LH or FSH, while GnRH was effective.

Summary: 1) PRL and GH responses to VIP are significantly blunted in MA patients when com pared to MI patients. 2) Similarly, PRL responses to TRH are significantly blunted in MA patient 3) VIP does not stimulate LH or FSH in either group. Conclusions: 1) The responses of prolact secreting pituitary tumors to VIP *in vivo* is similar to their responses *in vitro*. 2) *In vi* hyporesponsiveness to VIP is another example of the differing clinical and PRL secreto characteristics of MA versus MI, suggesting the possibility of a different pathogenesis.

● THE EFFECT OF SORBITOL AND ACTIVATED CHARCOAL ON SERUM THEOPHYLLINE CONCENTRATIONS FOLLOWING SLOW-RELEASE THEOPHYLLINE. MJ Goldberg,* R Spector, GD Park,* GF Johnson,* and P Roberts,* Departments of Internal Medicine, Pharmacology, Pharmacy, and Pathology, University of Iowa and the Veterans Administration Medical Center, Iowa City, IA.

Overdoses of slow-release theophylline preparations may result in the prolonged absorption of theophylline. Although the oral administration of multiple doses of activated charcoal (AC) will decrease the absorption of slow-release theophylline and increase theophylline clearance from the body, we studied whether the addition of the cathartic sorbitol to an AC regimen would further decrease serum theophylline concentrations. Nine healthy male volunteers received Theo-24 1200 mg/70 kg orally on three separate occasions after an overnight fast. On each occasion at 6, 7, 8, 10 and 12 hours after Theo-24 ingestion, each subject received in a randomized design either 300 ml of (a) water, (b) 20 gm AC in water, or (c) 20 gm AC in water plus 75 ml of 70% sorbitol at 6 and 8 hours only. The area under the serum theophylline concentration-time curve (AUC) from 6 to 30 hours after Theo-24 ingestion during the water, AC, and AC plus sorbitol phases were 305±16, 113±6 and 85±10 mg-hr/L (mean±S.E.) respectively (AC versus AC plus sorbitol, $p<0.01$; paired t-test [two-tailed]). The serum theophylline concentrations following AC plus sorbitol were significantly lower than following AC alone at 12, 15, 18, 21, and 24 hr. We conclude that the addition of sorbitol to an oral regimen of multiple doses of AC decreased the serum theophylline concentrations following therapeutic doses of slow-release theophylline to a greater extent than did the AC regimen alone.

● THE CIRCULATING GROWTH HORMONE BINDING PROTEINS: PARTIAL PURIFICATION AND STRUCTURAL CHARACTERIZATION BY AFFINITY CROSSLINKING. G Baumann and MA Shaw.* Northwestern University Medical School, Chicago, IL.

We recently described a specific, high affinity, low capacity binding protein (BP) for human growth hormone (hGH) in human blood (J Clin Endocrinol Metab 62:134, 1986). In addition to this main BP, hG also binds to a second, less well-characterized component of higher M in plasma. To gain further information on the molecular nature of these hGH-BP, we chemically crosslinked the hGH-BP complex to permit electrophoretic characterization. Whole plasma or a partially purified BP preparation - obtained by affinity chromatography on an hGH column - was incubated with ^{125}I-hGH, followed by crosslinking of the resulting complexes with 1 mM disuccinimidyl suberate. The reaction mixtures were then analyzed by SDS-PAGE at pH 10 with or without sulfhydryl reduction, and by non-denaturing PAGE at pH 10. Gel slabs were dried and autoradiographed. The bands representing specific crosslinks were identified by their absence when binding was carried out in the presence of excess (10 μg) unlabeled hGH. In SDS-PAGE two specifically labeled components of MW 76±7 kD and 124±6 kD (mean±SEM) were detected. Sulfhydryl reduction did not significantly alter the mobility, configuration or intensity of these bands. Ferguson plots in non-denaturing PAGE yielded a similar MW for the smaller of the two complexes. The use of whole plasma or purified BP yielded identical results.

Conclusions: Two circulating hGH-BP's can be identified in human plasma. Both are single-chain proteins and bind hGH to form complexes of apparent MW 76 and 124 kD. Based on the previously demonstrated 1:1 binding stoichiometry, the two BP's have calculated MW's of 55 kD and 100 kD, respectively.

● TIMING OF AMPHOTERICIN B THERAPY IS A CRITICAL DETERMINANT OF TOXICITY. KM Skubitz, MR Wick,* AP Skubitz,* RS Olshefski,* R von Roemeling,* TR Langevin,* RB Sothern,* and WJM Hrushesky. University of Minnesota Medical School, Minneapolis, MN.

Amphotericin B is the only efficacious treatment available for serious systemic fungal infections but is extremely toxic with a very narrow therapeutic index. Since the toxicities of a variety of drugs have been shown to be circadian stage-dependent, we examined the effects of the time of drug administration on the toxicity of amphotericin B in mice. Chronic i.p. amphotericin B administration at 1 of 3 test times demonstrated a circadian-stage dependence on survival. When percent lethality was analyzed at 50% overall survival, only 1/8 injected at 00 hours after lights on (HALO) was dead, while 10/10 mice injected at 16 HALO had succumbed; survival was intermediate for those treated at 08 HALO ($X^2 = 15.48$, $P = 0.005$). Post-mortem examinations revealed acute tubular necrosis as well as evidence of granulocyte and platelet aggregates in many microvascular systems. Acute i.p. administration of amphotericin B (35 mg/kg) resulted in 62% overall mortality with all deaths occurring within 4.6 days of treatment. A 2 X n chi-square analysis at 50% overall survival showed a significant time-effect ($X^2 = 12.63$, $p < 0.05$). At this stage only 4/15 mice injected at 02 HALO were dead whereas only 2/15 mice treated at 14 HALO survived. Thus, both the acute and chronic toxic effects of amphotericin B had a similar circadian stage dependence on time of drug administration. We conclude a similar randomized clinical trial is warranted in humans, since much of the toxicity of this efficacious but toxic drug may be alleviated by appropriate timing of drug administration.

● CIRCULATING MOLECULAR VARIANTS OF GROWTH HORMONE IN CHILDHOOD. G Baumann, RJ Winter,* and MA Shaw.* Departments of Medicine and Pediatrics, Northwestern University Medical School, Chicago, IL.

We recently identified several molecular forms of human growth hormone (hGH) in human plasma. They include the 22,000 dalton form (22K), the 20,000 dalton variant (20K), one or more acidic forms (deamidated, acylated or cleaved), and a series of corresponding oligomers up to pentameric hGH. These various molecular forms can be demonstrated in plasma after both pharmacological and physiological stimuli; their relative proportions are largely stimulus-independent. However, to date all data have been obtained in adult man. Since hGH exerts its most important biological function in childhood, examinati of circulating hGH forms in children is of particular interest. Accordingly, we have analyzed plasma hGH forms in 14 normal prepubert children, aged 7-13 years, at random times and 60 minutes after L-dop stimulation. hGH was extracted from plasma samples by immunoadsorben chromatography, and the extracts were analyzed by PAGE under native conditions at pH 7.5 as previously described for adult plasma. The electrophoretic patterns showed the same three monomeric hGH forms as those recognized in adult man, in similar proportions. The 22K form accounted for 67.9±4.5% (mean±SEM), 20K for 16.3±1.8%, and acidic hGH for 10.3±0.7% of total hGH. These values are not statistically different from those seen in adult plasma, with the exception of a marginally higher value for acidic hGH in children ($p \approx 0.05$). No evidence for previously unrecognized hGH forms was obtained.

Conclusions: The hGH forms secreted during childhood are qualitatively the same as those released by the pituitary in adult man. Their relative proportions in the circulation are also similar during both stages of life.