3) protein-G affinity chromatography to identify presence of PRL-antibody complex and, 4) samples negative for antibody by step (3) were applied onto a lectin concanavalin A column to examine the presence of glycosylated PRL forms. Analysis was according to manufacturer's instructions. All study samples and their chromatography fractions were measured using highly sensitive ELISA (DuoSet, R&D Systems, MN). Samples identified above as containing different mPRL forms were analyzed using four immunoassay analyzers in routine clinical use, namely; Advia Centaur® and Atellica® (Siemens, PA), Alinity-ci (Abbott Laboratories, IL), and Cobas 6000® (Roche Diagnostics, IN). **Results:** A total of 13 samples were entered into the study. PRL levels ranged from 21.4 to 1,469 ng/mL (median 48.8). Samples positive for mPRL (n=8) (with <40% recovery by PEG) exhibited predominant (52.3 to 95.0%) PRL activity in the (H) range (≥150kDa), with significant but relatively lower amount (3.6 to 34.1%) in the (M) range (≥30<150kDa) and (1.4 to 34.5%) at the (L) range (<30kDa). Samples negative for mPRL exhibited little PRL activity (1.2 to 5.1%) in (H) range, predominantly (60.0 to 79.4%) in the (M) range and moderate presence (15.4 to 38.9 %) in the (L) range. Two samples indeterminate for mPRL contained prolactin forms at all molecular weight levels, (H) (7.9, 27.1%), (M) (67.0, 40.7%), and (L) (5.9, 51.4%). Samples with mPRL exhibited significant binding to protein G affinity column indicating presence of PRL-antibody complex. Samples with (M) range mPRL forms exhibited significant lectin affinity binding. Samples with (H) mPRL showed markedly high PRL by two of the analyzers (Atellica®, Abbott Laboratories) and Cobas 6000®, (Roche Diagnostics). The deviation was more marked when using the Atellica® compared to Cobas 6000®. Samples with (M) mPRL showed marked deviation using the Roche Cobas 6000® compared to the others. In conclusion: Macroprolactin is heterogenous with antibody-PRL complex and aggregated glycosylated forms. Those forms exhibit variable immunoassay reactivity in different routinely used assays. Knowledge of circulating form as well as of their immunoreactivity is important when suspecting false hyperprolactinemia due to macroprolactin.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Memory Improving Effects in Social-Isolated Aged Rats by Seaweed Glycoproteins

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Social isolation and loneliness that could induce cognitive decline are serious public health problems in elderly. The trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) is a critical process in long-term potentiation of synapses that is necessary for memory formation. We previously found that glycoproteins of an edible seaweed *Capsosiphon fulvescens* (*C. fulvescens*) prevent aging-induced cognitive impairment via regulation of brain-derived neurotrophic factor in hippocampus. This study was to investigate whether chronic administration of hydrophilic glycoproteins of *C. fulvescens* (Cf-hGP) prevent cognitive dysfunctions caused by social isolation in aged rats and this effect is regulated by post synaptic density protein 95 (PSD-95)-mediated AMPAR trafficking and the glycosylation of Cf-hGP. Social isolation for four weeks decreased phosphorylation of extracellular signal-regulated protein kinase 1/2 (ERK1/2), PSD-95 (Ser295) and AMPAR GluR1 (Ser845) and increased expression of metabotropic glutamate receptor 5 (mGluR5) in synaptosome of the dorsal hippocampus, which was attenuated by chronic Cf-hGP treatment (15 mg/kg/day, 4 weeks). Blockade of mGluR5 abolished decrease in ERK1/2-mediated phosphorylation of PSD-95 (Ser295) and GluR1 (Ser845) in the socially isolated rats. In particular, chronic Cf-hGP treatment enhanced binding of ERK1/2 to PSD-95 and upregulated the surface movement of AMPAR GluR1 in the dorsal hippocampus. In addition, Cf-hGP treatment prevented spatial memory impairment caused by the social isolation, which was attenuated by inhibition of ERK1/2 or deglycosylation of Cf-hGP. These findings suggest that Cf-hGP-induced clustering of ERK1/2-mediated PSD-95 in the dorsal hippocampus improves memory formation in socially isolated aged rats and protein glycosylation contributes to enhancing the Cf-hGP effect.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Mice With Targeted Deletion of ARC Kisspeptin Exhibit Immature Gametogenesis and Impaired Fertility

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Hypothalamic kisspeptin is primarily synthesized in two discrete nuclei - the anteroventral periventricular (AVPV) and the arcuate (ARC) nuclei. We have previously developed a selective, conditional ARC kisspeptin knock-out (KO) mouse line, namely the Pdyn-Cre/Kiss$^{fl/fl}$ KO mice, that exhibited normal puberty onset in both sexes, but impaired estrous cyclicity and LH pulsatility in Pdyn-Cre/Kiss$^{fl/fl}$ KO females. To examine the end-organ effect of the lack of ARC kisspeptin, we examined gametogenesis, gonad morphology, and fertility. Hematoxylin and eosin (H&E) staining of serial-sectioned whole ovaries demonstrated that Pdyn-Cre/Kiss$^{fl/fl}$ KO female mice lacked corpora lutea - their ovarian folliculogenesis did not progress beyond antral follicle development, suggesting an ovulatory defect in Pdyn-Cre/Kiss$^{fl/fl}$ KO females. 75% of the Pdyn-Cre/Kiss$^{fl/fl}$ KO male mice had testes exhibiting a striking decrease in mature sperm in the seminiferous tubules. The remaining 25% showed evidence of mature sperm. Further evidence of a hypogonadal phenotype of the Pdyn-Cre/Kiss$^{fl/fl}$ KO mice included the significantly low weight and small size of the ovaries, uteri, and testes when compared to control littermates. In a controlled, continuous mating paradigm with proven WT males, 2-4-month-old Pdyn-Cre/Kiss$^{fl/fl}$ KO female mice failed to become pregnant or produce any pups, whereas age-matched WT females exhibited

normal pregnancies to term. Thus, Pdyn-Cre/Kiss$^{fl/fl}$ KO females have complete infertility. Ongoing studies of male fertility data suggest that Pdyn-Cre/Kiss$^{fl/fl}$ KO males are subfertile, in accordance with their variable spermatogenesis phenotype - some KO males sired pups when paired with proven, WT females, whereas other KO males are infertile. Future experiments include assessing the capability of Pdyn-Cre/Kiss$^{fl/fl}$ KO mice to respond to chronic, exogenous kisspeptin and GnRH administration to rescue abnormal LH pulsatility and estrous cyclicity in females, as well as the impaired fertility in both sexes.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

MIF Inhibition Suppresses Cell Viability and Induces Apoptosis via the ATF4-CHOP Pathway in Mouse Pituitary AtT-20 Cells

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Cushing's disease (CD) is characterized by cortisol overproduction due to ACTH hypersecretion from a pituitary tumour (PT). With an incidence of approximately 1.2 to 2.4 cases per million per year, CD patients have higher rates of morbidity and mortality than the general population. Surgical management is currently the first therapeutic option. However, remission rates vary between studies, and patients may suffer from complications caused by hormonal abnormalities from remnant PT tissues, the surgery itself, as medical treatment options are limited. Macrophage migratory inhibitory factor (MIF) is a cytokine expressed in various tumors, including ACTH-producing PTs, and has been found to play a crucial role in tumorigenesis. Previous studies demonstrate that MIF regulates cell growth via the signal transducer and activator of transcription 3 (STAT3) pathway, the mammalian target of rapamycin (mTOR) pathway, and autophagy. Together, these indicate MIF as a potential therapeutic target for PTs. However, the role of MIF in ACTH-producing PTs remains unknown. Using mouse ACTH-producing PT cells, AtT-20 cells as a model, we established that MIF overexpression led to increased cell growth. In contrast, pharmacological MIF inhibition by 4-iodo-6-phenylpyrimidine (4-IPP) and (S,R)-3-(4-Hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid (ISO-1) and genetic MIF downregulation by siRNA both suppressed cell viability and induced apoptosis, suggesting an anti-apoptotic role of MIF. Genetic MIF downregulation also increased the expression of apoptosis-inducible genes such as activating transcription factor 4 (ATF4) and C/EBP homologous protein (CHOP), and reduced ACTH production. However, pharmacological MIF inhibition had no effect on ACTH production, which suggests that the mechanism of pharmacological MIF inhibition may be different from MIF downregulation. Neither MIF upregulation nor downregulation affected cell signalling pathways such as the STAT3 pathway, the mTOR pathway, or autophagy. Our findings suggest that MIF inhibition can be a viable therapeutic approach for ACTH-producing PTs.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Mild Maternal Undernutrition Results in a Premature Neonatal Leptin Surge that Promotes Resistance in Male Offspring to a High Fat Diet

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Malnutrition alters leptin signaling, resulting in dysregulation of pituitary somatotropes that may be sex-specific. We reported sex differences in acute fasting responses with reductions in serum leptin in males, but not females (1). Maternal undernutrition can also alter the neonatal leptin surge by blunting with 50% food deprivation (2), causing a premature surge with 30% food deprivation (3), and resulting in metabolic dysfunction in adulthood (2,3). We developed a milder undernutrition model to relate more closely to society's nutritional challenges and to test the hypothesis that a shift in the neonatal leptin surge would result in sex-specific metabolic changes. Compared to paired *ad libitum* (Fed) dams, we studied pups from undernourished dams that were calorically restricted by 20% (CR20) from embryonic day 15 until postnatal day (PND) 21.

We tested 216 offspring from 11 Fed dams and 13 undernourished dams (CR20), detecting a leptin surge in control fed progeny at PND11. CR20 offspring of both sexes had an early surge (PND8) that was 62% ($P<0.0001$) higher compared to offspring of Fed dams and was maintained at high levels until PND11. Interestingly, GH levels at PND1 were 354% ($P<0.0001$) higher in the CR20 progeny compared to controls. By PND16, serum IGF-1 levels in underfed pups were lower than levels in control pups (111364±71 vs 244145±135 pg/ml; $P=0.0277$). CR20 male weights were 13.1% lower ($P<0.0001$) and lengths were 8.4% shorter ($P=0.0002$) than controls by 8 weeks of age and did not recover. CR20 female weights were lower by 11% ($P=0.0013$) and lengths were shorter up to 3 weeks of age.

At 3 months of age, offspring were exposed to a 45% HFD for 16 weeks, testing 54 pups from 3 dams per nutrient status. Fed mice from both sexes responded to the HFD with an average weight gain of 12.3g ($P<0.0001$) in females and 12.6g ($P<0.0001$) in males. Females from CR20 dams also gained weight (8.74g, $P<0.0001$) on the HFD, but was significantly lower than females from Fed dams ($P=0.0362$). Surprisingly, male progeny from CR20 dams did not respond to weight gain by HF feeding when compared to control fed males, appearing to be protected from impact.

Sex-specific changes in pituitary *Gh*, *Ghrhr*, and *Ghsr* mRNA levels were as follows. Among CR20 males exposed