The Effect of a Special Herbal Tea on Obesity and Anovulation in Androgen-Sterilized Rats (44494)

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Abstract. **A special herbal tea has been used to treat clomiphene-resistant anovulatory disease and obesity effectively, especially in polycystic ovary syndrome (PCOS) cases with hyperinsulinemia. The effect of the herbal tea on obesity and anovulation was investigated in androgen-sterilized rats (ASR). The ASR model was established by subcutaneous injection of 1.25 mg testosterone propionate to Sprague-Dawley female rats at the age of 9 days. Rats were sacrificed around 112 days of age. ASR manifested with PCO, anovulation, high food intake, elevated body weight, and obesity. Immunocytochemistry demonstrated that estrogen receptors (ER) were predominantly distributed in the cytoplasm of neuropeptide Y (NPY)-containing neurons in the preoptic area (POA), and the coexpression was also found in the nuclei and fibers of NPY-synthesizing neurons in the arcuate nucleus (ARC). Compared with that in normal control rats, NPY expression was increased, the numbers of ER in hypothalamic ARC-median eminence (ME) decreased, gonadotropin-releasing hormone (GnRH) levels in ME was decreased, serum estrogen (E_2) and leptin were elevated, and follicular stimulating hormone (FSH) and luteinizing hormone (LH) levels were reduced significantly in ASR. Significantly negative correlations between NPY and ER or GnRH, and between leptin and FSH or LH were observed. A positive correlation existed between serum leptin and body weight. These metabolic-endocrine changes in ASR were normalized after feeding the herbal tea. Both obesity and hypogonadotropin were expressed in ASR. The abnormal ovarian hormone milieu (elevated E_2 levels) may have enhanced NPY expression and resulted in less GnRH and gonadotropin secretion. The herbal tea reduced body weight and induced ovulation in ASR.** [P.S.E.B.M. 2000, Vol 223]

Fertility in mammals requires adequate nutrition and a reservoir of metabolic fuel (1). Extremes of body mass are associated with disturbance of reproductive function in women. High incidences of oligo- or amenorrhea and infertility are common in obese women or in women with a low percentage of body fat, such as trained distance runners, ballet dancers, and patients with anorexia nervosa (1, 2). Although the maintenance of reproductive function in adults is physiologically coupled with nutrition and energy, the nature of this linkage at the cellular and molecular levels remains unknown.

Leptin, a protein product of the *ob* gene secreted from adipose tissue, is a signal of the level of food intake and energy balance to the brain (3, 4). The genetically obese *ob/ob* mice (primary leptin deficiency) are characterized by hypogonadotrophic-hypogonadism, hyperinsulinemia, insulin resistance, infertility, and elevated hypothalamic neuropeptide Y (NPY) mRNA expression. These symptoms are reminiscent of those observed in patients with polycystic ovary syndrome (PCOS) (5–7). NPY, a 36-amino acid peptide, is one of the most potent food intake–stimulatory compounds and is widely distributed in the central nervous system (CNS) where it modulates the release of other neuropeptides and neurotransmitters (8, 9). NPY fibers establish synaptic contacts with the cell bodies and fibers of gonadotrophin releasing hormone (GnRH) neurons in the hypothalamic and basal forebrain regions (10). NPY is in-

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timately involved in the modulation of GnRH and luteinizing hormone (LH) secretion, *via* the regulation of GnRH release from nerve terminals in the median eminence (ME) and the facilitation of the LH response to GnRH. The direction and degree of NPY's interaction with GnRH are dependent on the steroid environment (9).

Jin Yu *et al.* (11) successfully modified the androgen-sterilized rat (ASR) model by injecting testosterone propionate into Sprague-Dawley female rats at the age of 9 days. ASR showed similar signs of hyperinsulinemia, hyperandrogenism, anovulation, infertility, increased body weight (BW), and obesity to PCOS in women and/or obese *ob/ob* mice (11–20). According to a theory in Traditional Chinese Medicine (TCM) and clinical study, a special herbal tea may be effectively used in the treatment of patients of PCOS with hyperinsulinemia (21, 22) and ASR (11–20). This paper explored the effect of this herbal tea on the regulation on serum estrogen and leptin levels, hypothalamic NPY and GnRH expression, and body weight and ovulation in the ASR model.

Materials and Methods

Animals and Treatments. Neonatal female Sprague-Dawley rats at 9 days of age were purchased from Experimental Animal Center of Shanghai Medical University (Shanghai, China). Fifty-five rats were injected subcutaneously (sc) with testosterone propionate (1.25 mg in neutral tea oil, equivalent to 0.05 ml, the 9th Pharmaceutical Factory, Shanghai) or 0.05 ml of neutral tea oil ($n = 15$). All rats were weaned at 21 days of life, housed in 25°C (50% humidity) with light from 0600 to 1800 hr, and food and water were available *ad libitum.* Vaginal smears were examined daily for 10 consecutive days from 70 days of age, and 40 out of 55 rats with persistent vaginal cornification (72.7%) were used as the ASR model; all rats in the control group showed cyclic estrus change. 1 ml/100 g BW of extract from a special herbal tea (for part of ASR, $n = 25$) or of distilled water (for normal rats, $n = 15$; and part of ASR, $n = 15$) was provided to rats from 80 until 101 days of age.

The special herbal tea was a recipe from Professor Jin Yu. It was composed of Cassia Bark (Latin Language, Cortex Cinnamomi Cassiae), Shorthorned Epimedium Herb (Herba Epimedii), Dodder Seed (Semen Cuscutae), Manyflower Solomonseal Rhizome (Rhizoma Polygonati), Chinese Fox-Glove Root (Radix Rehmanniae), Prepared Lateral Root of Aconite (Radix Aconiti Lateralis Praeparata), and others. The voucher specimens were deposited in the Department of Natural Medicine, Pharmaceutical School of Shanghai Medical University. The air-dried herbs were pulverized and extracted by refluxing three times with 95% ethanol. The extracts were concentrated under reduced pressure with rotavapor (BüCHI Rotavapor R-114, Switzerland). The extracted solvent was evaporated to give 342 mg/ml residue (equivalent to 3 g/ml of crude herbs).

Daily vaginal smears were monitored for 11 days after herbal tea treatment was terminated. After herbal tea treatment, those ASR without cyclic estrus changes were withdrawn from the study.

Food consumption over 24 hr was measured from 70 to 100 days of age in all rats.

Rats were weighed (no fasting) and perfused transcardially with 200–300 ml of 0.1 *M* phosphate-buffered saline (PBS, pH 7.4), followed by 400–500 ml of ice-cold fixation solution (4% paraformaldehyde in 0.1 *M* phosphate buffer, PB) under sodium pentobarbital anesthesia (50 mg/ml, intraperitoneally) at ≈ 112 days of age. Normal controls and ASR treated with herbal tea were sacrificed in the afternoon of proestrus. Blood was collected from the inferior vena cava before perfusion and stored at – 20°C for radioimmunoassays (RIA). The retroperitoneal white adipose tissues (WAT) and the ovaries of all rats were removed and weighed after fixative perfusion. Brains were removed and postfixed overnight in the same fixative solution containing 20% sucrose at 4°C. These brains were then transferred to 30% sucrose in 0.1 *M* PB until they sank. All experiments were performed in compliance with the Animal Experiments Guidelines and Animal Care of Shanghai Medical University.

Frozen sections of the brain (30-μm thickness) were cut coronally through the preoptic area (POA) and hypothalamus with a freezing microtome (Leica 820, Jung Histocut, Leica Microsystems Heidelberg GmRH, Neuenheimer, Heidelberg, Germany) according to a standard atlas described by Paxions and Watson (23). The sections were kept in a cryoprotectant solution (0.1 *M* PB, pH 7.4, containing 30% sucrose, 1% polyvinyl pyrrolidone, and 30% ethylene glycol) at – 20°C until further processing.

Single-Label Immunohistochemistry. Free-floating sections were processed for immunohistochemistry with affinity-purified rabbit antisera directed against ER (diluted to 1:400, Santa Cruz Biotechnology Inc., California, DA), NPY (1:8000, Sigma Chemical Co., St Louis, MO), and GnRH (1:1000, generously provided by Professor Zhuang LZ, the State Key Laboratory of Reproductive Biology, Science of Academy, Beijing, China, 1998), respectively. The primary antisera were localized by using the avidin-biotin complex system (ABC) with a commercially available kit (ABC Staining Kit, Santa Cruz). The sections were mounted onto gelatin-coated slides (Dako Corp., Carpinteria, CA, Denmark). Slides were then dehydrated and coverslipped with mounting medium.

Immunohistochemical controls included substitution of the first antibody with normal rabbit serum and omission of the first and/or the second antibodies.

Double-Label Immunocytochemistry. For the double fluorescence study, the sections were incubated in a mixture of a mouse antiserum directed against ER_α (diluted to 1:400, Santa Cruz) and a rabbit antiserum against NPY (1:8000, Sigma). The antibody mixtures were diluted in PBS containing 1.5% normal goat serum (NGS), 2% normal swine serum (Dako), and 0.3% Triton X-100. The antisera cocktails were incubated at 37°C for 2 hr, then at 4 °C for 48 hr. After washing in PBS containing 0.3% Triton X-100, the sections were incubated in a mixture of affinity-purified secondary antibodies: goat antimouse IgG conjugated with rhodamine (diluted to 1:20, Roche Molecular Biochemicals – Boehringer Mannheim, Indianapolis, IL, Germany) and swine antirabbit IgG conjugated with fluorescein isothiocyanate (FITC, diluted to 1:40, Dako). The mixture of secondary antibodies was diluted in PBS containing 0.3% Triton X-100 and 0.5% bovine serum albumin (BSA, w/v, Sigma) (pH 7.4) and incubated for 60 min at 37°C. The sections were rinsed and mounted with PBS before being air dried and coverslipped with buffered glycerol mounting medium (Dako), then preserved in the dark at 4°C for analysis.

The negative controls involved substitution of the mixture of first antibodies with the mixture of normal mouse serum and rabbit serum (Dako) that was diluted to match the concentration of the corresponding antibody and/or removing the ER and NPY antibodies from the staining protocol.

Hormone Assays. Circulating estrogen (E_2) and leptin concentrations were measured in duplicate with a commercial E_2 RIA kit (Diagnostic Products Corp., Los Angeles, CA) and rat leptin RIA kit (Linco Research Inc., St. Charles, MO). The intra- and interassay coefficients of variation were 5.2% and 9.1% for E_2 and 7.4% and 8.6% for leptin, respectively. The limit of sensitivity was 5 pg/ml for E_2 RIA, and 0.5 pg/ml for leptin RIA. The RIA reagents for rat LH and FSH were kindly provided by National Institute of Diabetes, Digestive & Kidney (NIDDK), and Dr. A. F. Parlow from the National Hormone and Pituitary Program (NHPP). The standard used for the FSH assay was rFSH-RP2, and the antiserum was anti-rFSH-S11. For LH the standard was rLH-RP3, and the antiserum was anti-rLH-S11. The tracers for both assays were purchased from New England Nuclear-Du Pont Research Products (Boston, MA). Final values were expressed as ng of rat LH or FSH per ml of serum. The within-assay coefficient of variation was 8.7% for LH and 9.6% for FSH.

Image Analysis. In ARC, 12 serial sections, 90 μm apart for each, were alternatively selected for the measurements of ER, NPY, and GnRH expression. These sections represented the entire rostrocaudal extent (Bregma −2.30 mm ~ −3.80 mm (23)) of these nuclei.

The immunoreactive (IR) densitometrical analyses were performed on a computer-assisted image-analysis system (Leica Wild MPS52, Leica Microscopy and Scientific Instrument Group, Heerbrugg, CH, Switzerland). Measurements of immunostaining intensity were taken in a matched field in each area after the images were threshold by density slicing to the same value. The mean background density (BACK) was used as a correcting factor. Integrated particle density (IPD, $\text{IPD} = \Sigma(\text{each pixel-BACK})$) and the total area of stained particles were used for calculating mean particle density (MPD), $\text{MPD} = \text{IPD}/(\text{total area of stained particles})$. The MPD measure provided a semiquantitative index of average staining intensity within a given region and was used as the unit of analysis. Preliminary counting for ER-IR carried out manually under a microscope (Olympus BH-2, Tokyo, Japan) gave values similar to those obtained by using this procedure. A similar approach for comparing immunostaining across brain regions was described by Yokosuka *et al.* (24).

For fluorescence double-labeled immunocytochemistry, sections through the ARC and the POA were examined with Confocal Laser Scanning Microscope (CLSM, Leica TCS-NT, Heidelberg, Germany) *via* dual channels fault scanning (0.9–1 μm thickness) and powerful 3D rendering and processing module.

Statistical Analyses. Group values were expressed as mean ± SD. Variables not normally distributed were log transformed before analysis by parametric methods. Data from immunohistochemistry, hormonal assays, and energy contents (BW, food intake, peritoneal WAT) were analyzed with Student-Newman-Keuls (snk) test using one-way analysis of variance (ANOVA). Relationships between serum leptin, hypothalamic NPY-IR, and independent variables in normal controls, ASR, and herbal tea–treated ASR were assessed with simple and/or multiple linear regression analysis, and Pearson (*r*) correlation coefficients were presented. Analysis of covariance (ANCOVA) and partial correlations were carried out to investigate the relationship between BW and serum leptin levels or the relative amount of hypothalamic NPY-IR, and the value of BW was used as a covariate. Differences were considered significant at the level of *P*-value less than 0.05. Statistical analyses were performed using the SAS 6.12 Package (Statistical Analysis System, SAS Inc., Cary, NC).

Results

Changes of Obesity and Anovulation in ASR. Daily food intake in the ASR was significantly greater than in the normal rats ($P < 0.01$) (Fig. 1). Body weights, retroperitoneal WAT, and serum leptin concentrations of ASR were significantly higher than those of normal rats ($P < 0.01$) (Table I).

Two consecutive estrous cycles were shown in all 15 control rats and in 15 out of 25 ASR treated with herbal tea (60%), whereas persistently keratinized vaginal cells were exhibited in all of 15 untreated ASR. Compared with normally developed ovarian follicles with 8~9 layers of granulosa cells in controls, the ovaries of ASR were characterized by cystic follicles with decreased numbers of granulosa cells (1~2 layers) and an absence of corpora lutea. All ovaries weighed 30%–50% less in ASR than in normal rats (31.24 ± 1.13 vs 67.54 ± 2.15 mg, $P < 0.01$) (11). The mean serum levels of LH and FSH were significantly attenuated in ASR compared with normal rats ($P < 0.01$) (Table I).

After herbal tea treatment, the food consumption, fat deposition, body weight, and serum leptin levels had normalized. Treatment with the herbal tea increased ovarian weight and the number of granulosa cell layers in ovarian follicles and corpora lutea were present in the ovaries of

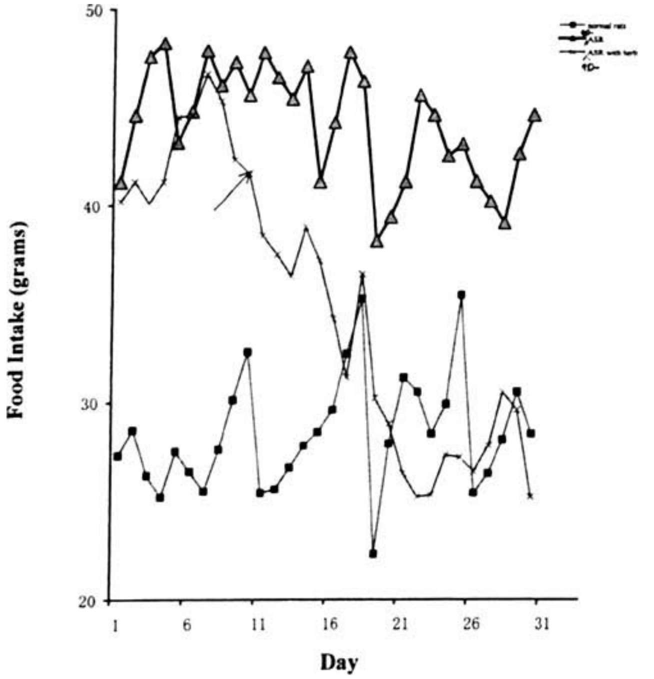

Figure 1. Food intake in normal rats, 9D-ASR, and 9D-ASR with herbal tea treatment. The food intake of all rats was measured daily from 70 to 100 days of life. 9D-ASR had higher food intake than normal rats ($P < 0.01$), and food consumption tended to decrease after treatment with herbal tea. Arrow indicated the initial day of herbal administration (80 days of age).

Table I. Body Weight (BW), Retroperitoneal WAT Weight, and Serum Hormone Concentrations in Normal Rats (C), 9D-ASR (A), 9D-ASR with Herbal Tea (H)

	C	A	H
Body weight (g)	209.5 ± 12.4	243.9 ± 8.3*	207.4 ± 14.3
Retroperitoneal WAT (g)	9.6 ± 0.7	17.9 ± 1.2*	10.2 ± 1.2
Estradiol (pg/ml)	65.57 ± 9.33	141.91 ± 24.97*	67.71 ± 7.13
Leptin (ng/ml)	1.19 ± 0.21	2.78 ± 0.16*	0.92 ± 0.28
FSH (ng/ml)	1.14 ± 0.13	0.25 ± 0.06*	1.06 ± 0.03
LH (ng/ml)	8.28 ± 0.87	1.17 ± 0.58*	8.05 ± 0.91

Note. Data were presented as mean ± SD ($n = 15$ in each group). *$P < 0.01$ compared with normal rats (C) (Student-Newman-Keus test). WAT, white adipose tissue.

treated animals (12). Gonadotropin levels did not differ between ASR with herbal treatment and normal rats ($P > 0.05$) (Table I).

Changes of ER in ARC-ME. Dense populations of ER-IR cells were observed throughout the rostrocaudal extent of the ARC. The total numbers of ER-IR nuclei of ARC were significantly lower ($P < 0.01$) and circulating E_2 levels higher ($P < 0.01$) in ASR than in normal control rats. After herbal tea treatment, ASR had similar densities of ER and serum E_2 levels to those of the control group ($P > 0.05$) (Fig. 2).

Changes of NPY in ARC-ME. In NPY-immunolabeled sections from control rats, numerous NPY-IR perikarya and nerve fibers with varicosities were shown throughout the rostrocaudal extent of the ARC and the internal zone of the ME. Beaded nerve fibers, preterminal axonal varicosities, and terminal-like structures formed a particularly dense network in the part of the ARC. The immunohistochemical staining of NPY in ARC was significantly denser in ASR than in normal rats ($P < 0.01$), which suggested that NPY innervation and production were more pronounced in ASR. There were no significant differences in the content of positive NPY immunostaining between ASR treated with herbal tea and control groups (Fig. 2).

Changes of GnRH in ME. GnRH-IR fibers were located predominantly in the internal zone of the ME. Compared with controls, ASR exhibited significantly less GnRH immunolabeling in nerve fibers throughout the ME ($P < 0.01$), which significantly increased to normal levels after herbal tea treatment (Fig. 2).

The Colocalization of NPY and ER in POA and ARC. Double immunofluorescence staining and a confocal microscope were used to visualize NPY-IR cells in POA in close apposition to ER-IR cells, and the results indicated that many NPY-IR neurons (especially in cytoplasm) were decorated with ER-labeled cells (Fig. 3A). A substantial degree of colocalization between axonal terminals derived from NPY neurons and ER-labeled cells was also detected in the ARC. The fibers of NPY neurons were also immunoreactive for ER (Fig. 3B).

Correlation Analysis. In linear regression analysis, serum leptin levels were negatively correlated with serum FSH and LH concentrations (Fig. 4). The inverse correlation was also observed between the relative amount of NPY-IR and ER-labeled nucleus, and between NPY-IR and GnRH-IR fibers in ARC (Fig. 5). To assess whether serum leptin levels correlated with hypothalamic NPY expression levels in this study, leptin levels were regressed on NPY variables with and without control for the potential confounding factor of BW in ANCOVA. In such analysis, serum leptin concentrations and central NPY levels were all positively correlated with BW (Figs. 6A and 6B); however, no significant association between serum leptin concentrations and NPY expression was detected either at control or ASR with herbal tea treatment after correcting with BW.

Discussion

In 1961, ASR was produced by Barraclough (25) by injection of testosterone propionate to female rats at 5 days of age (5D-ASR). The 5D-ASR model was characterized by multiple ovarian follicular cysts, an elevation of serum androgen levels, and anovulation, experimental changes that are similar to symptoms associated with human PCOS. As a result, the 5D-ASR animal has been used as a model for PCOS (25–27). In the last decade, 5D-ASR had been considered to be invalid as a model of PCOS by other investigators (28). Since 1981, for the purpose of exploration of

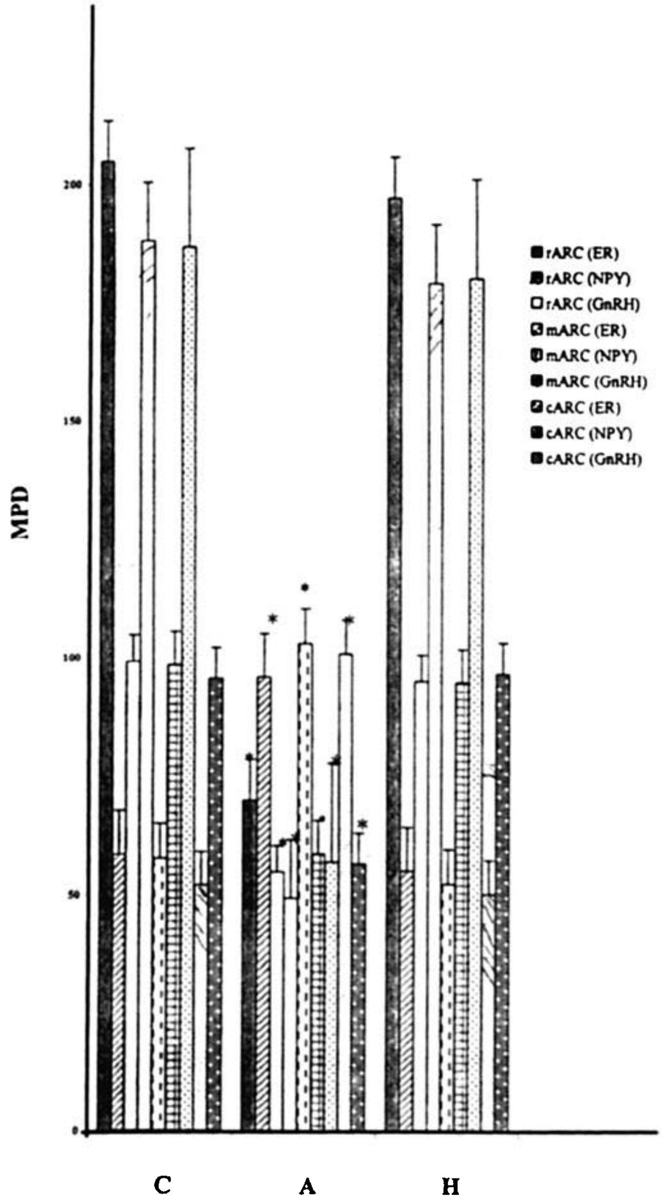

Figure 2. The relative amounts of immunoreactive (IR) ER, NPY neurons and fibers, GnRH fibers terminals of the rostrocaudal extent of ARC-ME in normal rats (C), 9D-ASR (A), 9D-ASR with herbal tea (H). Data shown were mean particle density (MPD, mean ± SD). Asterisks indicated a statistically significant difference ($P < 0.05$) compared with the values for the respective control (Student-Newman-Keus test). For example, MPD of GnRH-IR fibers of rostral ARC-ME in 9D-ASR (open bars) were lower than those in normal rats or 9D-ASR fed with herbs ($P < 0.05$), and no significant difference was found between Group C and H ($P > 0.05$). r, m, c ARC, rostral, medial, caudal arcuate nucleus and median eminence; ER, estrogen receptor; NPY, neuropeptide Y; GnRH, gonadotrophin releasing hormone.

herbal function on reproduction, Jin Yu had modified this model by changing the testosterone injection date to rats that were 9 days of age (9D-ASR), and proved this rat as a model with hyperinsulinemia, hyperandrogenemia, hypogonadotropic anovulation, high androgen receptor (AR) mRNA expression in pancreas, and obesity, which was 60% affected in ovulation by a clinically effective herbal tea (11–20). Moreover, in the current study, 9D-ASR exhibited hyperphagia, higher storage in adipose tissue, obesity, dysregulation of the circulating levels of leptin (the adipocyte-derived hormone), reduced numbers of hypothalamic ER, overactivity of ARC-ME NPY neurons, and decreased immunoexpression of GnRH fiber terminals in the ME.

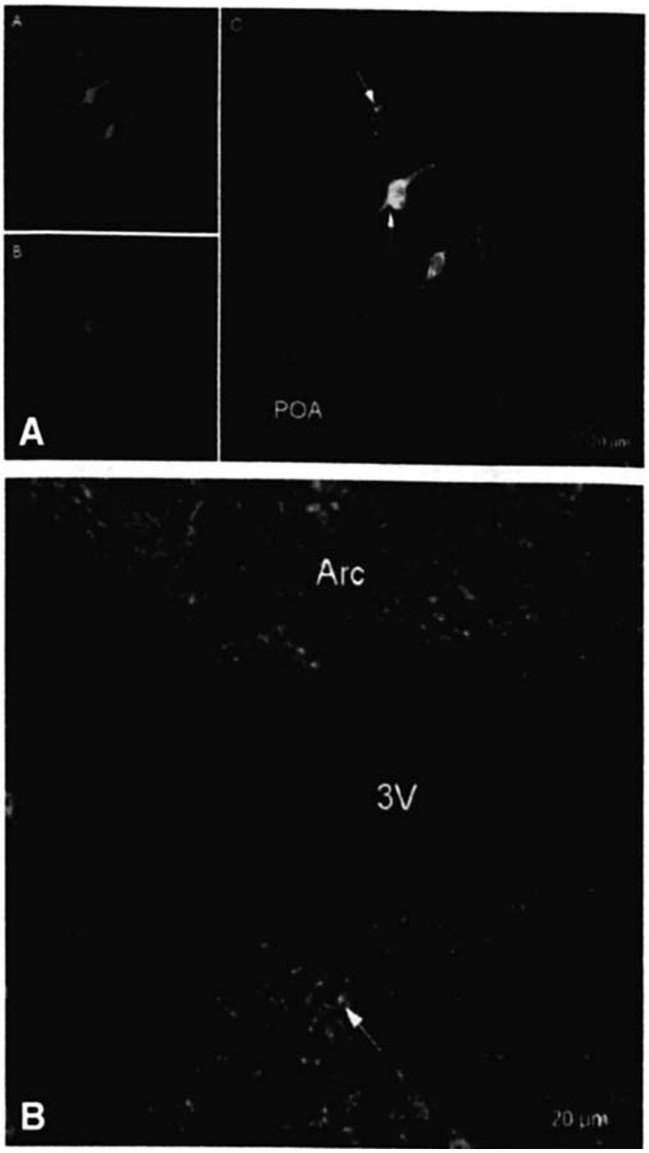

Figure 3. Fluorescence double-labelling immunocytochemistry and confocal images to show the relationship between estrogen receptor (ER) immunoreactive (IR) cells and neuropeptide Y (NPY)-IR neurons or fibers (A) in the region surrounding the preoptic area (POA) and (B) in the medial part of the arcuate nucleus of the hypothalamus. Images showed possible contact or coexpression (C, yellow) between NPY-containing neurons (A, green) or fibers (A, green) and ER-IR (B, red). ER might distribute in cytoplasma or nucleus or fibers (arrows) of NPY-containing neurons. 3V, third ventricle. Scale bar, 20 µm.

The overexpression of NPY mRNA and enhanced NPY release in the ARC-paraventricular nucleus (PVN) may contribute to the obesity in Zucker (*fa/fa*) rats or *ob/ob* mice (29–31). The present studies suggest that obesity in 9D-ASR may be related to the overactivity of central NPY-synthesizing neurons in ARC. NPY has also been reported to either stimulate or inhibit GnRH-LH release, and this bimodal response is obviously dependent upon the gonadal

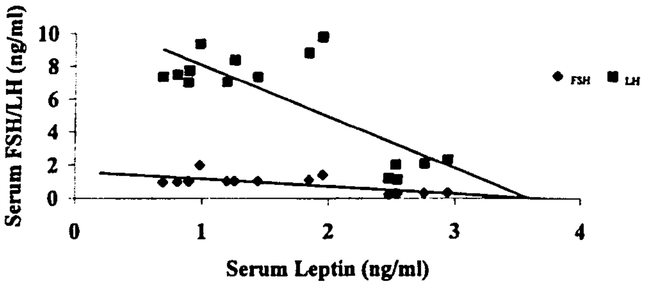

Figure 4. Correlation between serum levels of leptin and FSH or LH in normal rats, 9D-ASR, 9D-ASR fed with herbal tea. Correlation coefficient (*r*) was -0.7517 for FSH or -0.8444 for LH in this assay.

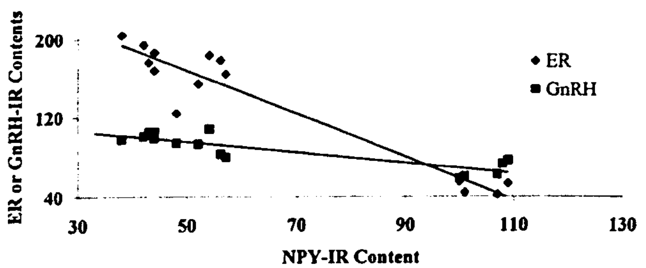

Figure 5. Correlation between immunoreactive contents of ARC NPY and ER or GnRH (data expressed as mean particle density, MPD) in normal rats, 9D-ASR, ASR with herbal tea. Correlation coefficient (*r*) was -0.7104 for ER and -0.8802 for GnRH in this assay.

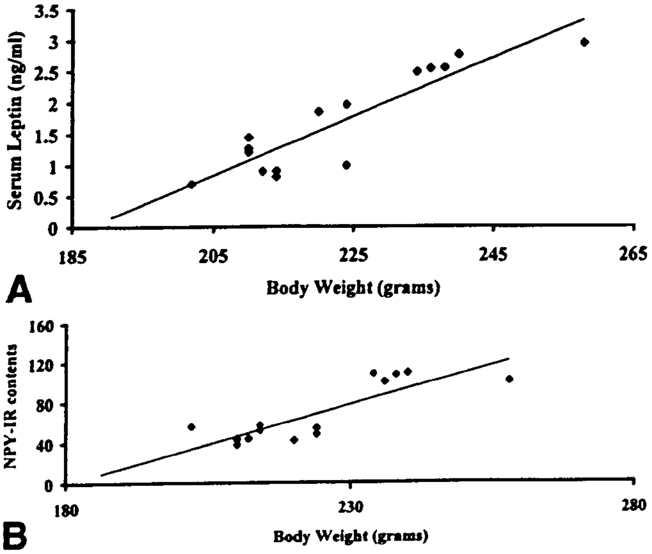

Figure 6A. Correlationship between body weight and serum leptin levels in all rats, i.e., normal rats, 9D-ASR, 9D-ASR treated with herbal tea. Correlation coefficient (r) was 0.8977 in this assay.
Figure 6B. Correlationship between body weight and the relative amount of hypothalamic NPY-IR (data expressed as mean particle density, MPD) in normal rats, 9D-ASR, and 9D-ASR fed with herbal tea. Correlation coefficient (*r*) was 0.8568 in this assay.

steroid milieu (32, 33). Intraventricular administration of NPY causes a suppression of LH release in gonadectomized rats (34, 35). Negative correlation between NPY values in ARC and GnRH values in ME in this study suggested that NPY may be one of the important neuropeptides in the neural regulation of GnRH. Thus central NPY may serve a dual purpose on energy metabolism and reproductive regulation in 9D-ASR.

With double immunofluorescence staining and confocal microscope examination, ER was found to be colocalized in the NPY neuron in the ARC and in POA, which may shed light on estrogen regulation of GnRH. In 9D-ASR, high serum estradiol levels might initiate a cascade of downregulating the amount of ER distributed in NPY-containing neurons in ARC, enhancing local NPY expression, decreasing GnRH release in ME, leading to low pituitary LH and FSH secretion, and resulting in anovulation. However, this remains to be clarified experimentally.

Leptin plays a major role in controlling body fat stores by altering feeding behavior (reducing appetite), metabolism (enhancing energy consumption), autonomic nervous system activity, and body energy balance in rodents, primates, and humans (36, 37). However, several lines of evidence suggest that leptin also has a significant effect on the reproductive system. First, leptin may reduce body weight, improve hyperinsulinemia and insulin resistance, and reverse hypogonadism in *ob/ob* mice (38, 39). Second, leptin treatment of normal mice can accelerate puberty (40, 41). We observed a negative correlation between levels of serum leptin and FSH/LH, and between levels of hypothalamic NPY and GnRH expressions in 9D-ASR. Unlike leptin deficiency in *ob/ob* mice, higher leptin levels are positively correlated with body weight, just like the positive correlation between hypothalamic NPY value and body weight in 9D-ASR. The expression of leptin and NPY appear to be reciprocally regulated (6, 42, 43), though there was no statistically significant correlation between serum leptin and ARC NPY expression levels when BW factor was adjusted in this study.

Dr. Jin Yu has been concentrating on the treatment of PCOS with integrated Western and traditional Chinese medicine (TCM) for more than 40 years. According to the theory of TCM and clinical study, the herbal tea was found to be effective in the treatment of patients with PCOS with clomiphene resistance and in 9D-ASR. Ovulation occurred in 58.9% of 33 patients (21), and most patients and 9D-ASR had decreased BW, serum insulin, and androgen levels (unpublished data, 1998, approved by Dr. Jin Yu).

In this study, after herbal treatment with 9D-ASR, a decrease in estrogen levels may have caused an elevation of hypothalamic ER in succession with falling hypothalamic NPY expression, which may have led to the elevation of GnRH and reduction of body weight. These data add neuroendocrine evidence to the peripheral effect of the herbal tea on obesity and anovulation.

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