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Short-term fasting attenuates the response of the HPG axis to kisspeptin challenge in the adult male rhesus monkey (*Macaca mulatta*)

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ABSTRACT

Aims: In primates, changes in nutritional status affect the hypothalamic-pituitary-gonadal (HPG) axis by still poorly understood mechanisms. Recently, hypothalamic kisspeptin-GPR54 signaling has emerged as a significant regulator of this neuroendocrine axis. The present study was designed to examine whether suppression of the reproductive function by acute food-restriction in a non-human primate is mediated by decreased responsiveness of the HPG axis to endogenous kisspeptin drive.
Main methods: Five intact adult male rhesus monkeys habituated to chair-restraint, received intravenous boli of human kisspeptin-10 (KP10, 50 μg), hCG (50 IU), and vehicle (1 ml) in both fed and 48-h fasting conditions. Plasma concentrations of glucose, cortisol and testosterone (T) were measured by using enzymatic and specific RIAs, respectively.
Key findings: The acute 48-h fasting decreased plasma glucose ($P<0.01$) and *T* ($P<0.005$) levels, and increased cortisol levels ($P<0.05$). KP10 administration caused a robust stimulation of *T* secretion in both fed and fasted monkeys. However, mean *T* concentration and *T* AUC after KP10 administration were significantly ($P<0.01$–0.005) reduced in fasted monkeys. Likewise, the time of the first significant increase in post-KP10 *T* levels was also significantly ($P<0.01$) delayed. *T* response to hCG stimulation was similar in fed and fasted monkeys.
Significance: The present results indicate that under fasting conditions the KP10 induced *T* response is delayed and suppressed. These data support the notion that fasting-induced suppression of the HPG axis in the adult male rhesus monkey may involve, at least in part, a reduction in the sensitivity of the GnRH neuronal network to endogenous kisspeptin stimulation.

Introduction

The hypothalamic-pituitary-gonadal (HPG) axis that controls reproduction is very sensitive to the level of metabolic fuels (Wade et al., 1996; Schneider, 2004; Wade and Jones 2004). Under fasting conditions, in which there is a deficit not only of different macro-nutrients necessary for normal development but also of calorie, an abnormal reproductive situation is observed (Kuderling et al., 1984; Badger et al., 1985; Cameron and Nosbisch 1991). A number of studies have shown that fasting induced suppression of reproduction is caused by a decrease in release of GnRH rather than by inhibition of pituitary-gonadal axis sensitivity to GnRH (Bergendahl et al., 1991; Cameron and Nosbisch 1991; Aloi et al., 1997). The master position of hypothalamic GnRH in the hierarchy of signals controlling the gonadotropic axis makes it a target of multiple regulators of both central and peripheral origin. In the context of the former, a wide array of excitatory and inhibitory neurotransmitters/neuropeptides have been identified (reviewed in Terasawa and Fernandez 2001; Plant and Shahab 2002; Plant and Barker-Gibb 2004; Ebling 2005), and it is likely that the fasting induced arrest of the HPG axis is mediated by modulation of inhibitory and /or excitatory afferent drives to the GnRH neurons. Such a possibility is strengthened by the observation of reduced sensitivity of HPG axis to NMDA administration in fasting (Shahab et al., 1997).

Recently, the kisspeptin-GPR54 system has been implicated as major regulator of neuroendocrine GnRH release on the basis of genetic and pharmacological studies (reviewed in Gottsch et al., 2006; Plant 2006; Tena-Sempere 2006). It is not only involved in the initiation of puberty (Han et al., 2005; Shahab et al., 2005), but also plays a major role in control of GnRH secretion in the adult (Dhillo et al., 2005; Ramaswamy et al., 2007). Recent evidences in rodents indicate a role for kisspeptin-GPR54 system in mediating metabolic cues on the HPG axis. Both fasting and lactation were shown to significantly reduce expression of hypothalamic *KiSS-1* mRNA (Castellano et al., 2005; Luque et al., 2007; Yamada et al., 2007) as well as *GPR54* mRNA (Luque et al., 2007). Therefore, the present study examined the hypothesis that, in higher primates, fasting-induced suppression of reproductive function involves a reduction in the

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kisspeptin drive to the HPG axis, by comparing the response of the HPG axis to exogenous KP10 administration in fed and fast adult male rhesus monkeys. The output of the HPG axis response was monitored by estimation of plasma *T* concentration. Plasma glucose and cortisol concentrations were also monitored to confirm that fasting had produced a metabolic deficit.

Material and methods

Animals

Five adult intact male rhesus monkeys (*Macaca mulatta*), 6–8 years old, weighing 6.5–9.3 kg were used in this study. The animals were housed in individual cages, under standard colony conditions in the Department's primate facility. The animals were fed daily with fresh fruits (0900–0930 h) hard boiled eggs at 1100 h and bread at 1300–1330 h. Water was available ad libitum. Appetite was monitored for a month prior to the beginning of the experiments. All animals ate their feed in 10–20 min. To reduce the effects of stress on blood sampling, animals were habituated to chair restraint several weeks prior to commencing of the experiments. The duration of restraint was gradually increased until a daily period of 3 h was attained. The animals were sedated with ketamine hydrochloride (Ketler, Astarapin, Germany 5 mg/kg BW im) for placement in and removal from the restraining chair. All experiments were approved by the Departmental Committee for Care and Use of Animals.

Venous catheterization

To permit sequential withdrawal of blood samples and *iv* administration of KP10, the animals were anesthetized with ketamine hydrochloride (10 mg/kg BW, im) and a teflon cannula (Vasocan Branule, 0.8 mm/22 G O.D, B. Braun Melsungen AG, Belgium) was inserted in the saphenous vein. The distal end of the cannula was attached to a syringe via a butterfly tube (Length 300 mm, volume 0.29 ml, 20 GX3/4″, JMS Singapore). Experiments were not initiated until the animals had fully recovered from sedation.

Pharmacological agents

Ketamine hydrochloride, heparin (Rotex media, Trittau, Germany) and human chorionic gonadotropin (hCG; Pregnyl®, N.V Organon Oss Holland) were purchased locally. Human kisspeptin-10 (112–121) was purchased from Calbiochem (La Jolla, CA, USA). Working solutions of KP10 were made in normal saline (0.9% NaCl).

Blood sampling

Sequential blood samples (2 ml) were obtained at 15 min intervals in heparinized syringes. Following withdrawal of each sample, an equal amount of heparinized (5 IU/ml) normal saline was administered. Blood sampling was conducted between 1500–1900 h i.e. starting about one and half hour after completion of daily feeding. Blood samples were centrifuged at 3000 rpm for 15 min, and plasma was separated and stored at −20 °C until hormones analysis.

Samples were collected at 60, 45, 30, 15 and immediately before administration of vehicle or KP10 at time 0, and sampling continued for 3 h after the injection at 15 min intervals. Studies of the effects of kisspeptin were conducted during a period of one month (February–March 2006) on 4 different days. The treatments were given in following order:

1: *Fed Vehicle (Fed V) Experimental Day 1:* Normal fed animals were administrated vehicle (normal saline; 1 ml, *iv*) as a control after taking 0 min sample.
2: *Fast Kisspeptin (Fast K) Experimental Day 2:* Five days following the first sampling regimen, the animals were deprived of all their meals on two consecutive days (48-h fasting) and bled as above. KP10 (50 µg/ml, *iv* bolus) was administered after collection of 0 min sample.
3: *Fed Kisspeptin (Fed K) Experimental Day 3:* Nine days after the 2nd blood sampling period, fed animals received KP10 as above.
4: *Fast Vehicle (Fast V) Experimental Day 4:* Five days after the 3 rd sampling period, 48-h fasted monkeys received vehicle as on Experimental Day 1.

Response to hCG

In order to determine whether fasting changes testicular responsiveness to LH stimulation, hCG (50 IU) was administered intravenously in the fed and fasting condition to three of the 5 monkeys. Blood samples were collected at 30 min intervals for 90 min following the injection of hCG immediately after taking a pretreatment sample at 0 min. hCG was given in April, 2008.

Radioimmunoassay (RIA) of testosterone and cortisol

Plasma *T* and cortisol concentrations were determined by using solid phase competitive RIAs. The *T* and cortisol RIA kits were purchased from Immunotech Marselle Cedex 9, France and Immunotech Prague 10, Czech Republic, respectively. The RIAs were performed as per the manufacturers instructions.

The sensitivity of the *T* assay was 0.025 ng/ml and intra- and inter-assay coefficients of variation were both <15%. Sensitivity of the cortisol assay was 0.82 µg/dl and the intra-assay coefficient of variation was <6%.

Assay of plasma glucose

Plasma glucose concentrations were measured using a Glucose pap kit (AMP Medizintechnik Graz, Austria) which employs glucose oxidase based enzymatic detection. 50 µl of glucose pap stable liquid was added to 10 µl aliquots of control, standards or plasma. Optical density at 550 nm was noted after 10 min of incubation. All measurements were completed within 30 min by using a photometer (UV-120-01, Shimadzu, Japan).

Statistical analysis

Statistical comparisons for the mean plasma levels of glucose, cortisol and *T* under fasting and fed conditions and for mean pre- and post-treatment *T* levels were made by paired Student's *t* tests. *T* responses to KP10 challenges under fed and fasting conditions were quantified in terms of area under the curve (AUC), calculated by the formula described by Abbud and Smith (1993). AUC were also compared in fed and fasted KP10 treated monkeys by *t* test. All data are presented as mean (±SEM). Results were considered statistically significant at $P<0.05$.

Results

Glucose and Cortisol levels in fed and fasting conditions

Mean plasma glucose concentrations during the 1 h pre-vehicle treatment period in fed animals were significantly higher ($P<0.01$) than those obtained in the same time period after 48-h of fasting (Fig. 1A). In contrast, mean plasma cortisol concentration were significantly increased ($P<0.05$) in 48-h fasted monkeys as compared to the fed monkeys (Fig. 1B).

Basal T concentrations in fed and fasting conditions

Effect of fasting on *T* secretion was analyzed in vehicle treated animals during the 1 h period before vehicle injection. (Fig. 2A). Mean basal plasma *T* concentrations in fed monkeys were significantly higher ($P<0.005$) compared to those in fasted monkeys (Fig. 2B).