The role of neuropeptides in the disturbed control of appetite and hormone secretion in eating disorders

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Abstract

OBJECTIVE: It has been reported that neuropeptides may play a role in the control of appetite and in the mechanism of hormone release.
Neuropeptides such as β-endorphin, neuropeptide Y (NPY), galanin and leptin may affect hormones release, on the other hand the hormonal status may modulate neuropeptide activity.
METHODS: The material consisted of 90 obese women, 30 women with Anorexia Nervosa, and 30 healthy, lean women of control group.
Plasma β-endorphin, NPY, leptin, somatostatin and serum pituitary and gonadal hormones concentrations were measured with RIA methods.
RESULTS: We observed the highest plasma NPY levels in obese hypertensive and diabetic patients. After carbohydrate administration (OGTT) a marked increase of insulin, β-endorphin and NPY was found. The blunted response of GH to GH-RH may be connected with increased somatostatin activity and hyperinsulinemia. The abnormal response of LH to opioid blockade may be a result of disturbed opioid and NPY activities in obese patients. However in patients with anorexia nervosa, plasma leptin and NPY concentrations were low. The disturbances in β-endorphin release are also observed.
CONCLUSIONS: The neuroendocrine disturbances in obesity and in anorexia nervosa are opposite. The feedback mechanism between leptin and NPY is disturbed in both in obesity and in anorexia nervosa. An abnormal activity of neuropeptides may lead to disturbed control of appetite and hormonal dysregulation in eating disorders.

Introduction

It has been that neuropeptides may play a role in the control of appetite and in the mechanism of hormone release [1–5]. Many factors are involved in the physiological mechanism of behaviour feeding β endorphin (β-E), neuropeptide Y (NPY) and galanin are orexigenic peptides and they regulate appetite by influence on hypothalamic appetite centres, thermogenesis and catecholamine activity [6, 7]. The activity of these peptides is modulate by other mediators (insulin, CRH, MSH, Serotonin, CART, orexines and cytokines (Il-1, Il-6, TNF) [6–18].

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ORIGINAL ARTICLE

The interaction between central and peripheral signals is due to leptin – a peptide secreted by adipocytes. [19]. Leptin and other neuropeptides may penetrate through blood brain barrier [20]. Leptin acts on central nervous system (CNS) through hypothalamic receptors and decreases expression mRNA NPY [21]. Neuropeptides such as β-endorphin neuropeptide Y (NPY), galanin and leptin may affect hormones release and on the other hand the hormonal status may modulate neuropeptide activity [22–26].

The aim of this study was to evaluate the relationship between hormonal and neuropeptides activity in eating disorders.

Material and methods

The material consisted of 90 obese women (BMI 30 – 40 kg/m ^{2}) mean age 30 yrs, 30 women with anorexia nervosa (mean age 20 yrs) and 30 healthy lean women (control group) – mean age 27 yrs.

Plasma β endorphin leptin, NPY, somatostatin, and serum LH, FSH, GH, PRL, estradiol, progesterone were measured with RIA methods.

Results

Plasma NPY levels in control group, obese patients, obese hypertensive subjects and obese diabetic patients were presented in Figure 1.

The highest concentrations of NPY we found in hypertensive and diabetic obese patients. After carbohydrate administration (OGTT) a marked increase of insulin, β-endorphin and NPY was observed in obese patients (figs. 2, 3, 4). The significant correlation between NPY and insulin was found in obese subjects (fig. 5). The blunted response of GH to GH-RH and abnormal response of LH to LH-RH to naloxone was presented in Figures 6 and 7.

In women with anorexia nervosa we observed a decrease of plasma NPY and leptin levels (Figs. 8, 9).

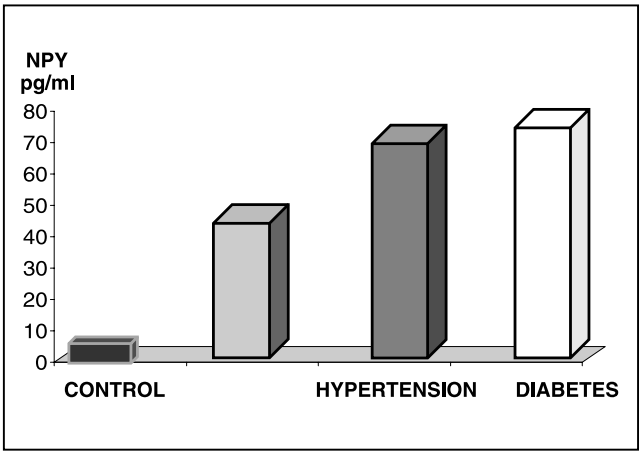

Fig 1. Plasma NPY concentrations in obese women (BMI 30–40 kg/m^2) in obese women with hypertension and in obese women with diabetes

Discussion

We previously published, that plasma leptin, NPY and galanin concentrations were increased in obese patients and they were correlated with BMI [7]. In this paper we found the highest levels of NPY in obese hypertensive and diabetic patients. A marked increase of insulin, β-endorphin and NPY after carbohydrate administration may suggest that NPY, β-endorphin in obese patients may originate from the pancreas and other sites of gut rather than from brain.

The significant correlation between NPY and insulin may indicate that NPY play an important role in the mechanism of insulin resistance in obesity. Some hormonal disturbances may appear in obese patients. Our previous data indicated that somatostatin release is exaggerated, however VIP release is decreased [27, 28]. The blunted response of GH to GH-RH may be connected with increased somatostatin activity and hyperinsulinemia [28]. The abnormal response of LH to opioid blockade with naloxone may be result of disturbed opioid and NPY activities in obese patients.

It has been established that anorexia nervosa (AN) according to the diagnostic criteria (The Diagnostic and Statistical Mannual of Mental Disorders – DSM–IV) characterised by:

1. behaviour directed at inducing a loss weight (deficit of body weight >25%)
2. amenorrhea for 3 consecutive months.

In the mechanism of hormonal disturbances in anorexia nervosa two important factors may be involved:

1. hypothalamo-pituitary dysfunction
2. disturbed peripheral hormonal metabolism

The disturbances in the control of appetite, thermoregulation, thirst and dysfunction in neurohormones release (GnRH, TRH, GHRH, gherlin, somatostatin (S-S) and CRH (corticotrophin releasing hormone) result from hypothalamo-pituitary dysfunction. The endocrine disturbances in AN concern hypothalamo-pituitary-adrenal, gonadal, thyroid, GH-somatomedin axes [5, 28]. Amenorrhea in anorexia nervosa is of hypothalamic origin.

The functional deficiency of GnRH is due to increased opioid, dopaminergic and melatonin activity and decreased in leptin and noradrenergic activity [29, 30]. The deficit of GnRH leads to lack of pulsatile secretion of LH, abnormal rhytm of LH and secondary to defficiency in estradiol and progesterone release [5].

In this study we found that plasma leptin and NPY concentrations were decreased in patients with AN. The feedback mechanism between leptin and NPY is disturbed.

The loss of β-endorphin (βE) circardian rhytm, increased nocturnal secretion of βE and blunted response of βE to clonidine and domperidone were also observed [29]. The exaggerated response of GH to GH-RH, TRH may be connected with decreased S-S and leptin activities and with increased activity of gherlin.

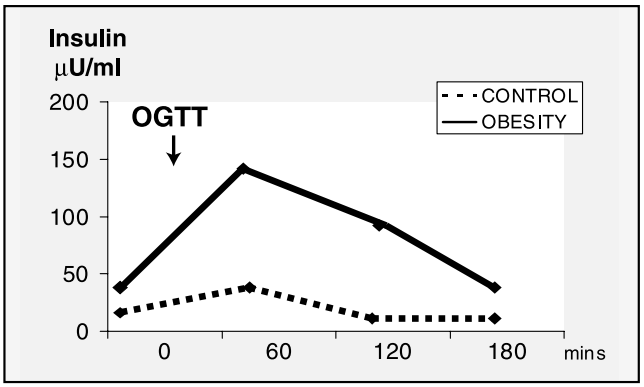

Fig 2. Plasma insulin concentrations after carbohydrate administration (OGTT) in obese women

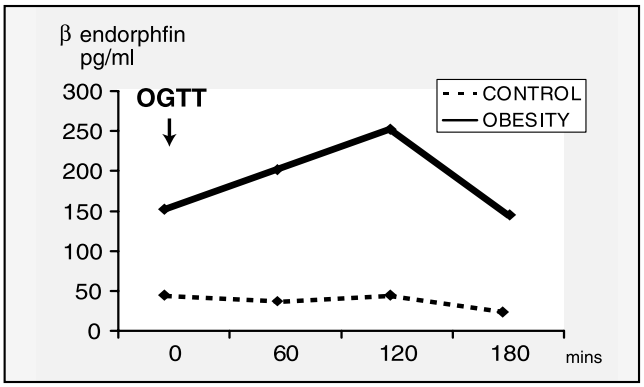

Fig 3. Plasma β-endorphin concentrations after carbohydrate administration (OGTT) in obese women

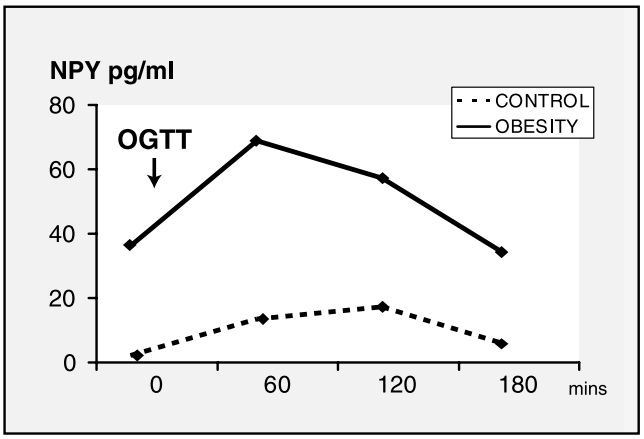

Fig 4. Plasma NPY concentrations after carbohydrate administration (OGTT) in obese women.

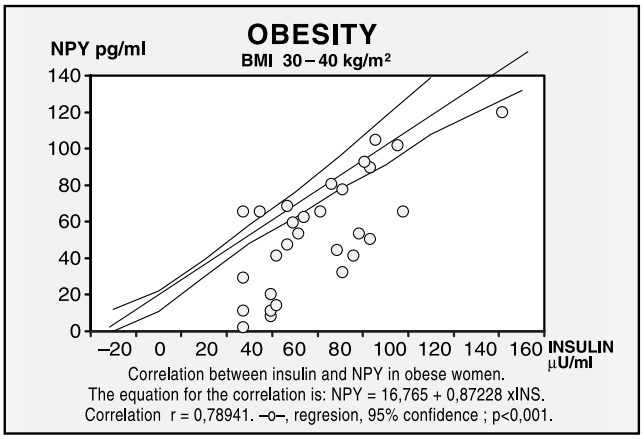

Fig 5. The concentration between NPY and insulin in obese women

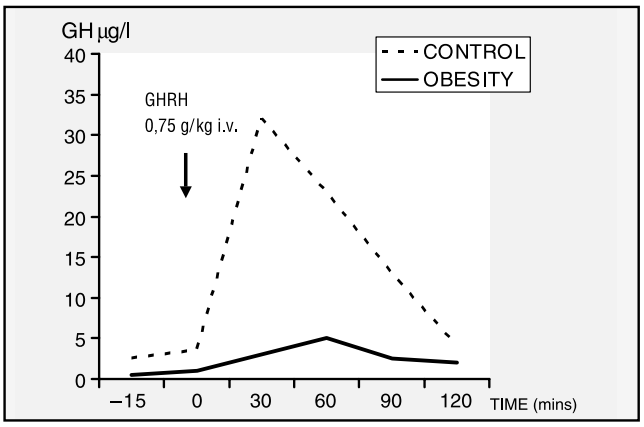

Fig 6. Serum GH concentrations in response to GH-RH injection in obese women

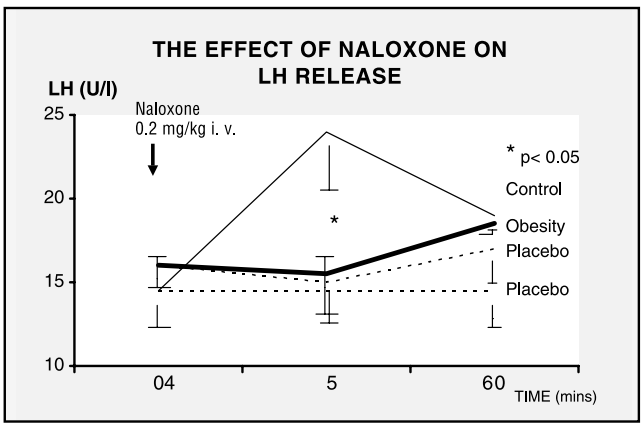

Fig 7. Serum LH concentrations in response to Naloxone in obese patients

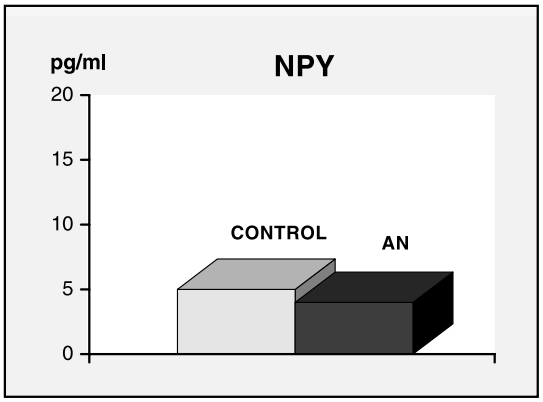

Fig 8. Plasma NPY concentrations in anorexia nervosa

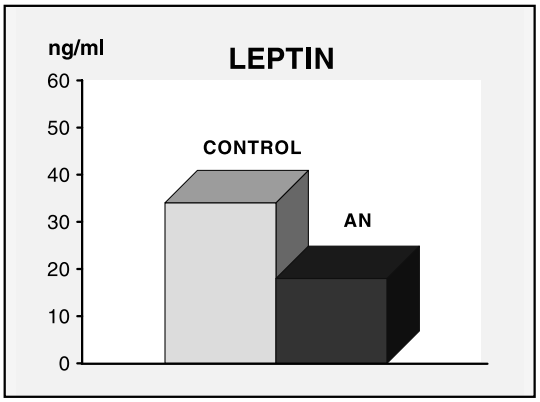

Fig 9. Plasma leptin concentrations in anorexia nervosa.

The disturbed peripheral hormonal metabolism is associated with starvation [29, 31, 32]. The several examples of changes in the hormonal peripheral metabolism are listed below.

1. The decreased peripheral conversion of T4 (thyroxine) to T3 (triiodothyronine) and the increase of revers T3.
2. The changes in metabolism of cortisol (the decrease of cortisol clearance leads to discrepancy between serum cortisol concentrations and its metabolites).
3. The changes in androgens metabolism (the increase of testosterone and the decrease of dehydroepiandrosterone in the dramatic period of weight loss).

Conclusions

1. The neuroendocrine disturbances are opposite in obesity and in anorexia nervosa.
2. The feedback mechanism between leptin and NPY is disturbed in both in obesity and in anorexia nervosa.
3. An abnormal activity of neuropeptides may lead to disturbed control of appetite and hormonal dysregulation in eating disorders.

Acknowledgement

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Abbrevations

AP-5: DL-2-Amino-5-phosphonovaleric acid
EAA: Excitatory amino acid
(+)MK-801: ((5R,10S)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine hydrogen maleate
NMDA: N-methyl-D-aspartate
PRL: Prolactin
RIA: Radioimmunoassay

Introduction

Glutamate, the major excitatory amino acid (EAA) in the brain, had been recognized to be involved in neuroendocrine regulation [1, 2]. In advance, the distribution of *N*-methyl-D-aspartate (NMDA) receptor subunit NR1 in hypothalamus and pituitary indicated their possible involvement in pituitary hormone release [3]. Various stressors and the exogenous NMDA receptor agonists could induce the prolactin (PRL) release [2, 4]. The NR1 was localized in the pituitary PRL cells in female rats [5], and glutamate could stimulate the PRL release *in vitro* in dispersed pituitary cells of female rats [6]. Moreover, NMDA receptor antagonist blocked the immobilization-induced PRL release in female rats at estrus, suggesting a mediatory role of NMDA receptors [7]. In male rats, however, the results were controversial as to the role of glutamate receptor in controlling PRL secretion [8–10], and it was still not elucidated that whether NMDA receptors were involved in PRL release in stress response [11].

The present study firstly investigated the role of NMDA receptors in restraint-induced PRL release in the adult male rat by using central or systemic administration of NMDA receptor antagonists, and secondly examined the effects of NMDA receptor blockade on the restraint-induced PRL release in peripuberal and middle-aged male rats.

Materials And Methods

Animals: Male rats of *Wistar* strain, purchased from the Imamichi Institute for Animal Reproduction (Ibaraki, Japan), were employed for experiment. After arrival, the rats were maintained on a 14L:10D cycle (lights-on 0500–1900 h) in a room at 22 ± 1 °C for at least two weeks, with food and water *ad libitum*. When used for the experiment, the rats were at the peripuberal (45-days, weighing 120–150g), adult (12-weeks of age, weighing 380–420 g) and middle-aged (16-months, weighing 650–700 g) stages, respectively.

Cannula implantation: At seven days before the restraint, the adult rat was implanted with a chronic brain cannula in the right lateral ventricle of the brain for intracerebroventricular (*icv*) injection. The lateral cerebroventricle was localized in accordance with the coordinates of Paxinos and Watson [12]. The cannula implantation was operated according to the reported method [13]. Briefly, the animal was anesthetized by pentobarbital (40 mg/kg, *ip*), and was placed in a stereotaxic frame (SR6, Narishige, Japan) using non-traumatic ear bars. Under aseptic conditions, a small hole was drilled in the skull overlying the right lateral ventricle, and a guiding cannula (22 G, C313G, PlasticOne Inc., USA) was lowered into right lateral ventricle (1.0 mm behind from *Bregma*, 1.5 mm right lateral to the midline, and 3.5 mm ventral to the surface of the cortex). The cannula was sealed in place using cold curing dental acrylic. After the operation, the rats were nursed for 6 hours before being sent back to the animal room. Furthermore, for intravenous injection and repetitive blood sampling, at two days before the restraint performance, a cannula was inserted into the right jugular vein under pentobarbital anesthesia (40 mg/kg, *ip*), and the vein cannula was tunneled under the skin of the back and closed.

Drug preparation: AP-5 (DL-2-Amino-5-phosphonovaleric acid), a competitive NMDA receptor antagonist and (+)MK-801 ((5R,10S)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine hydrogen maleate), an uncompetitive NMDA receptor blocker, were purchased from Sigma. Before using, the drug was dissolved in 0.9% saline to reach the working concentration. The *p*H of the solutions was adjusted to 7.0 with 1 *N* NaOH.

Restraint: At the day of trial, early in the morning, the brain cannula and the vein cannula were extended, and every available device was taken to minimize the possible disturbance on the animal. At 15 minutes before the restraint (–15 min), 10 μl solution of AP-5 (50, 100 μg /rat) or MK-801 (50 μg/rat) for treatment group, or 10 μl of sterile saline for control animals, was respectively injected through the brain cannula in 1.5 min. Then the animals were restrained in a plastic restrainer (DecapiCones Restrainer DC-200, Braintree Scientific Inc., USA) for three hours. At -15 min, 0 min (immediately before the restraint), and 15, 30, 60, 120 and 180 min during the restraint respectively, 0.5 ml of blood sample was collected through the vein cannula. A 0.5ml of blood sample was taken at –15 min before the antagonist solution (AP-5 in a dose of 10 mg/kg; MK-801 in a dose of 5 mg/kg; 37 °C) or sterile saline (as control, 37 °C) was infused into vein through the cannula. Blood samples were collected in chilled, heparinized tubes and were centrifuged at 1700 *g* ×30 min at 4 °C. The plasma was stored at –30 °C till hormone assay.

In peripuberal group or middle-aged group, the rat was implanted with a jugular vein cannula 2 days before restraint as described above. The rat was treated with MK-801 (5 mg/kg, *iv.*) 15 min later the rats were restrained according to same protocol for the adult rat.

RIA of prolactin: Concentration of PRL in plasma was measured using NIDDK kit for rat PRL. Hormone for iodination was rat PRL-I-5. The antiserum used was anti-PRL-S-9. Results were expressed in term of NIDDK rat PRL-RP-2.

Statistics: All data from RIA analysis were statistically evaluated with one-way ANOVA followed by Dunnet's multiple *t*-test. Difference between means was considered statistically significant if $p<0.05$.

Fig. 1. MK-801 (50 μg/rat, icv) prevented the restraint-induced prolactin release at 15 min in adult male rats. AP-5 (I: 50 μg/rat; II: 100 μg/rat; icv) partially suppressed the PRL release at 15 min. Values were presented as means±S.E.M; n=8-11. Symbols a, b and c show significance at p<0.05, denoting the statistically significant difference from the basal level before stress (a), from the time-matched control (b), and from the peak level (c), respectively.

Fig. 2. MK-801 (5 mg/kg, iv) prevented the restraint-induced prolactin release for 3 h in adult male rats. AP-5 (10 mg/kg, iv) did not modify the PRL response. Values were presented as means±S.E.M; n=8-10. Symbols a, b and c show significance at p<0.05, denoting significant difference from the basal level before stress (a), from the time-matched control (b), and from the peak level (c), respectively.

Fig. 3. MK-801 (5 mg/kg, *iv*) prevented the restraint-induced prolactin release for 3 h in peripuberal male rats. Values were presented as means±S.E.M; n=6-7. Symbols a, b and c show significance at $p<0.05$, denoting significant difference from the basal level before stress (a), from the time-matched control (b), and from the peak level (c), respectively.

Fig. 4. MK-801 (5 mg/kg, *iv*) prevented the restraint-induced prolactin release for 3 h in middle-aged male rats. Values were presented as means±S.E.M; n=6-7. Symbols a, b and c show significance at $p<0.05$, denoting significant difference from the basal level before stress (a), from the time-matched control (b), and from the peak level (c), respectively.

Results

Adult rats

As shown in Fig. 1, the restraint elicited a dramatic increase of plasma PRL in the adult rat (control) at 15 min. The PRL level declined at 30 min, and then gradually decreased, but was still higher than baseline by the end of the restraint. Neither *icv* AP-5 nor *icv* MK-801 alone influenced the basal level of PRL. Both doses of AP-5 significantly suppressed the restraint-elicited PRL release at 15 min compared with control ($p<0.05$), however the higher dose of AP-5 (100 μg/rat) produced a gradual decline of PRL after 15 min, while in the lower dose group (50 μg/rat) the decline was observed after 30 min. In the rat pre-administered with MK-801 (50 μg/rat), the restraint-caused PRL release was significantly suppressed.

Neither AP-5 nor MK-801 injection (*iv*) alone affected the PRL basal level. At all time points during restraint, AP-5 (*iv*) failed to significantly modify the PRL response to restraint (*vs.* control). But MK-801 treatment prevented the PRL response, and PRL level decreased gradually to below baseline at 120 and 180 min (Fig. 2).

Peripuberal rats

In the peripuberal rat (Fig. 3), the PRL basal level and the PRL response to restraint in control group were similar to that in the adult rat. MK-801 treatment (5 mg/kg, *iv*) did not caused a change of

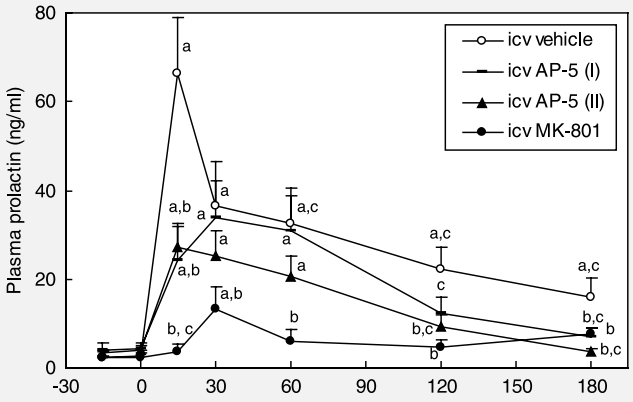

Fig. 1.

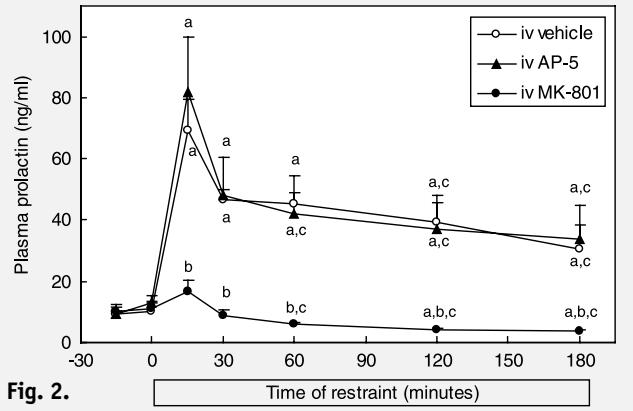

Fig. 2.

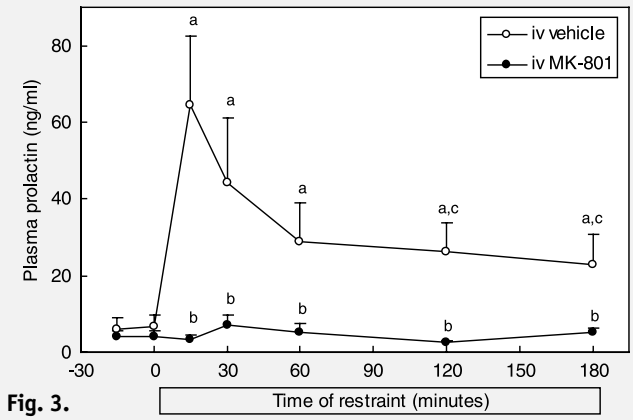

Fig. 3.

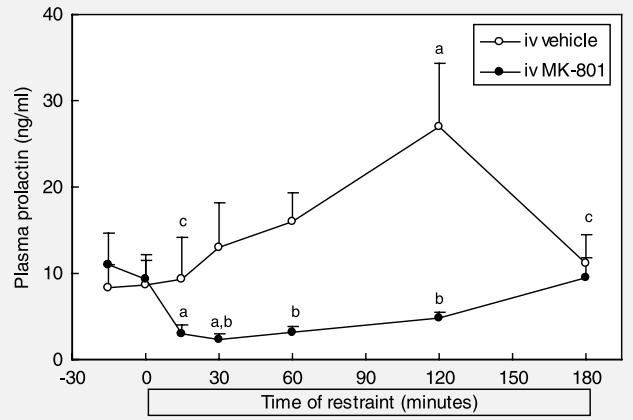

Fig. 4.

the basal level of PRL, but prevented the PRL release at all setting time points during restraint.

Middle-aged rats

In the middle-aged rat (Fig. 4), the PRL increased slowly during the restraint and reached a peak at 120 min. At 180 min, the PRL level returned to the baseline. The application of MK-801 alone (5 mg/kg, *iv*.) did not significantly produced a change of the PRL basal level. But a significant decrease of PRL was observed at 15 and 30 min of the restraint ($p<0.05$) and then the PRL recovered slowly to the basal level by the end of the restraint.

Discussion

In this study, we found that treatments with AP-5 and MK-801caused an inhibitory effect on restraint-induced PRL release in male rats (Fig. 1), that indicated that central NMDA receptors were involved in the PRL release. MK-801 showed more potency in inhibiting the PRL response than AP-5 did. Such a functional difference might be due to the different pharmacological mechanisms of the two antagonists: AP-5 functions by competing the binding sites with endogenous NMDA receptor agonists, but MK-801 blocks the activated ion channel [14–16].

Due to its polar molecule structure, AP-5 could poorly pass through the blood-brain barrier (BBB), but MK-801 readily penetrated the BBB [14–16]. The above results explained why AP-5 (10 mg/kg, *iv*) failed in blocking restraint-induced PRL response when was treated, suggesting the affect was performed through a hypothalamic mechanism (Fig. 2). In addition, this drives us to consider that NMDA receptors in PRL cells in pituitary, if there were any, might not participate in the restraint-activated pathway linking to PRL release. Nevertheless, MK-801 blocked the restraint-induced PRL release. It was documented that hypothalamic paraventricular nucleus (PVN) mediated restraint-induced PRL in the male rat [17], and that an immobilization stress induced significant increase of NMDA receptor subunit NR1 mRNA in neurons of the hypothalamic PVN and supraoptic nucleus [18]. Thus the hypothalamic PVN might be a candidate for the action site of NMDA receptors in restraint-induced PRL response. In view of that the PRL release was controlled by the prolactin-releasing factors (PRFs) and prolactin-inhibiting factors (PIFs) [4], MK-801 might have prevented the PRL response either by suppressing PRFs or by stimulating PIFs, or by both. Since tuberoinfundibular dopaminergic neurons were reported not to be involved in the restraint-induced PRL release in male rats [19], more consideration should be taken on PRFs actions. A hypothesis was proposed concerning the NMDA receptor involvement in immobilization-induced PRL release in female rats at estrus [7]. However, the mechanism for male rat is far from elucidated [4].

In contrast to our study, Zelena et al showed that MK-801 alone failed to modulate PRL release induced by immobilization [10]. This controversial might be due to different administration protocols and stressors.

Peripuberal male rat has developed a mature mechanism for PRL response to stress [20], as also manifested in our study (Fig.3). The potency of MK-801 in blocking PRL response in the peripuberal rat indicates that the NMDA receptors play an important role in mediating restraint-evoked PRL release at the peripuberal stage.

In the middle-aged male rat, restraint induced a delayed response of PRL with smaller magnitude (Fig. 4), compared to that in peripuberal and adult rats. This delayed response might be related to the lowered content of glutamate in hypothalamic and/or the blunted PRL response to NMDA receptor agonists [21], as well as the decrease in density of NMDA receptors with aging [22]. The blunted magnitude of PRL response might be due to the decreased pituitary PRL mRNA during aging in the male rat [23]. Therefore it might not be surprising that MK-801 produced different modulation on PRL response in the middle-aged rat.

Conclusion

In summary, NMDA receptors are involved in restraint-induced release of PRL in the male rat, probably by the action in the hypothalamus rather than in the pituitary. Their function might be different in the middle-aged male rat from in peripuberal and adult male rats.

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