NMDA receptor antagonists reduce restraint-induced release of prolactin in male rats

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Abstract

OBJECTIVE: To investigate the effects of *N*-methyl-D-aspartate (NMDA) receptor antagonists on restraint-induced release of prolactin (PRL) in male *Wistar* rats of different ages.

DESIGN: Rats were implanted with a brain ventricular for icv injection, and with a jugular vein cannula for iv injection. Competitive NMDA receptor antagonist AP-5 and noncompetitive NMDA receptor blocker MK-801 was injected via brain cannula or vein cannula and was restrained for 3 hours. The blood sample was collected through vein cannula during the restraint. The plasma concentration of prolactin was measured by RIA.

RESULTS: The restraint-induced PRL release in the adult rat (12-weeks) was significantly suppressed by MK-801 (50 μg/rat, *icv*; or 5 mg/kg, *iv*) and was partially inhibited by pretreatment of AP-5 (50, 100 μg/rat, *icv*), but was not changed by systemic administration of AP-5 (10 mg/kg, *iv*). MK-801 (5 mg/kg, *iv*) prevented the restraint-induced PRL release in the peripuberal rat (45-days) and in the middle-aged rat (16-months).

CONCLUSION: Central NMDA receptors mediate restraint-induced PRL release in the male rat.

ORIGINAL ARTICLE

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 $22 \pm 1 ^\circ$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; $37 ^\circ$C) or sterile saline (as control, $37 ^\circ$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 $4 ^\circ$C. The plasma was stored at $–30 ^\circ$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.

Fig. 1.

Fig. 2.

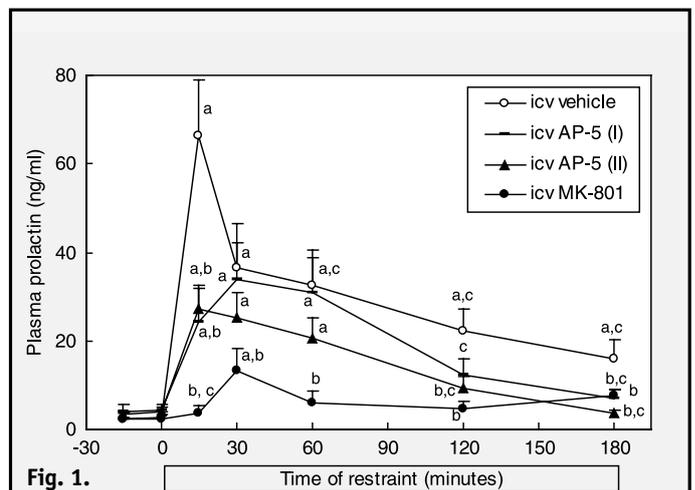**Fig. 3.**

Fig. 4.

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).
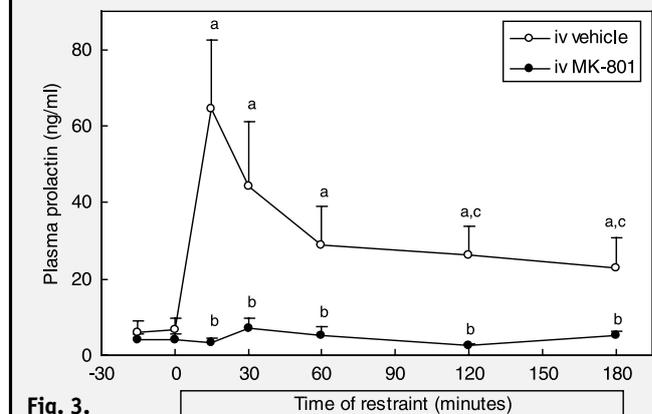
Peripuberal rats

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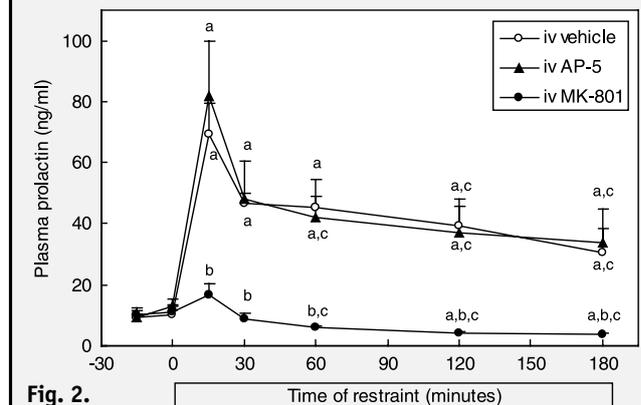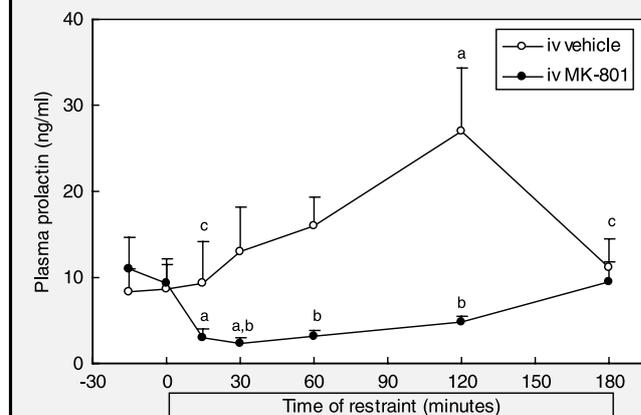
the basal level of PRL, but prevented the PRL release at all setting time points during restraint.

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.

Acknowledgements

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