Effects of N-methyl-D-aspartic acid and kainic acid on prolactin secretion in hyper- and hypoprolactinaemic conditions

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Abstract

Objective: The stimulatory and inhibitory effects of N-methyl-D-aspartic acid (NMDA) and kainic acid on prolactin (PRL) secretion have been correlated with the serum prolactin concentrations before drug administration. In the present experiments, we analysed the role of NMDA and kainic acid in PRL secretion in females with different serum concentrations of PRL.

Methods: Hypoprolactinaemic females were obtained by ovariectomy or after administration of diethyldithiocarbamate (an inhibitor of dopamine-ß-hydroxylase). Chronic hyperprolactinaemia was induced by neonatal administration of testosterone or oestradiol and acute hyperprolactinaemia was induced either by administration of α-methyl-p-tyrosine (an inhibitor of tyrosine hydroxylase) or by ether exposure. To analyse the role of dopamine in the effects of NMDA, we measured pituitary concentrations of dopamine after NMDA treatment and the effects of pretreatment with domperidone.

Results: (1) NMDA, but not kainic acid, stimulated PRL release in cyclic females. This effect was independent of serum PRL concentrations and was not accompanied by a decrease in pituitary concentrations of dopamine. (2) NMDA did not change PRL secretion in neonatally androgenized females, whereas NMDA and kainic acid inhibited PRL release in neonatally oestrogenized females. The inhibitory effects of NMDA and kainic acid were blocked by domperidone. (3) Kainic acid inhibited PRL secretion in prepubertal hyper- and hypoprolactinaemic rats. (4) Hyperprolactinaemia induced by stress was counteracted by administration of NMDA and kainic acid.

Conclusions: (a) NMDA has a dual effect on prolactin secretion that is independent of prior prolactin concentrations and of dopamine activity, but kainic acid is only inhibitory. (b) The stimulatory or inhibitory effects of NMDA and kainic acid on PRL secretion were not strictly related to basal PRL concentrations and necessarily involved a change in the secretion of prolactin releasing factors, as no correlations were observed between changes in pituitary concentrations of dopamine and serum PRL concentrations. (c) Females rendered hyperprolactinaemic by neonatal administration of testosterone or oestradiol responded differently after NMDA administration. (d) NMDA and kainic acid blocked the mechanisms involved in stress-induced PRL secretion.

Introduction

In the past few years, growing evidence has indicated the crucial role of excitatory amino acids in the regulation of pituitary secretion (1–3). Their action is mediated by the activation of different subtypes of postsynaptic receptors, which include N-methyl-D-aspartate (NMDA) receptors, kainate receptors, 2-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid receptors, amino-4-phosphobutyrhy acid receptors and metabotropic receptors. NMDA and kainate receptors are activated specifically by N-methyl-D-aspartic acid (NMHA) or kainic acid and antagonized by MK-801 (4) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) (5).

NMDA and kainic acid have dual effects on prolactin (PRL) secretion in female rats. Stimulatory effects of NMDA have been reported in adult female rats (6, 7), whereas both stimulatory (8) and inhibitory actions (9) have been described in prepubertal animals. It has been proposed that the effects of NMDA on PRL depend on the previous circulating concentrations of the hormone, with a conversion of the PRL response from stimulation in cyclic females to inhibition during lactation (10, 11).

We have reported previously that NMDA and kainic acid decreased PRL secretion in normoprolactinaemic male and female rats through an increase in release of dopamine (9, 12, 13). We hypothesized that the effects of NMDA and kainic acid on PRL release are not dependent on basal PRL secretion. The present experiments were carried out to analyse the effects of NMDA and kainic acid on PRL secretion in hyperprolactinaemic experimental conditions induced by the administration of steroids to neonatal or adult rats (14–16), by ether stress (17, 18) or after administration of...
α-methyl-β-tyrosine (α-MPT, an inhibitor of tyrosine hydroxylase) (19). In addition, the effects of NMDA and kainic acid in females with low concentrations of PRL, induced by ovariectomy or administration of diethylthiocarbamate (DDC, an inhibitor of dopamine-β-hydroxylase). We conclude that the inhibitory effects of NMDA and kainic acid on PRL secretion were not dependent on prevailing PRL serum levels.

Material and methods

Animals and drugs

Female Wistar rats born in our colony were used. The day on which animals were born was considered as day 1 of age. At that time, the litter size was adjusted to eight pups per dam, and the animals were injected with oestradiol benzoate or testosterone propionate (100 μg or 1.25 mg respectively, dissolved in 0.1 ml olive oil) or vehicle. The animals were weaned at 21 days of age, housed in groups of four or five per cage and maintained under controlled conditions of temperature (20°C) and light (12 h light : 12 h darkness) with free access to pelleted food (Panlab, Barcelona, Spain) and tap water. After vaginal opening, vaginal smear cycles were monitored daily. Only control females showing regular cycles were used. Oestrogenized females showed persistent oestrous vaginal smears, whereas the vaginal membrane was closed in androgenized females. Animals were studied at around day 90, the control rats being in metoestrous. In experiment 6, females were killed on day 30. All the experiments started at 1000 h and special precautions were taken to avoid non-specific stress.

NMDA, kainic acid, DDC and α-MPT were supplied by Sigma (Barcelona, Spain) and the NMDA antagonist, MK-801, and the dopaminergic antagonist, domperidone, were supplied by Research Biochemicals International (RBI, Natick, MA, USA). The kainate antagonist, DNQX, was obtained from Tocris Neuramin (Essex, England). All the substances were dissolved in saline immediately before use; DNQX was suspended in saline after gentle shaking, and domperidone was dissolved initially in a few drops of methanol and thereafter in saline to the final concentration used. Previous reports had demonstrated the effectiveness of NMDA, kainic acid (9, 12, 13, 20, 21) and MK-801 (22) in doses similar to those used in the present experiments.

The guidelines of the Research Committee of Córdoba University on animal use were followed.

RIAs

After centrifugation, serum was collected, frozen, and stored at −20°C until required for use. The prolactin concentrations were measured in duplicate in 25–50 μl aliquots using a double-antibody method and RIA kits supplied by NIH (Bethesda, MD, USA). Rat-Prl-I-6 was labelled with iodine-125 by the chloramine T method (24). Hormone concentrations were expressed as ng/ml of the reference preparation, Prl-Rat-RP-3. The intra-assay coefficient of variation was 9% and the interassay variability was 12%. Sensitivity was 10 pg/tube.

Dopamine determinations

Pituitaries were sonicated in 1 ml mobile phase (aqueous solution of 100 mmol/l formic acid, 0.33 mmol/l octane sulphonic acid, 1 mmol/l citric acid, 0.10 mmol/l EDTA, 5% methanol and 0.25% diethylamine, adjusted to pH 3.1 with KOH). After centrifugation at 75 000 g for 20 min at 4°C, the supernatants were filtered through 0.22 μm pore filters at 54 g for 20 min at 4°C. The HPLC system consisted of a Beckman System Gold Series with a 116 pump, a 7215 S injection valve equipped with a 20 μl sample loop and an NEC system controller (Beckman Instruments Inc., San Ramon, CA, USA), a BAS LC-4B amperometric detector with a glassy carbon transducer kit (Bioanalytical Systems Inc., West Lafayette, IN, USA) and a Merck LC, C-18 reverse phase, 5 μm, 125 × 4 mm column (Merck, Darmstadt, Germany). Flow rate was 1 ml/min. The concentration of dopamine in the samples was determined by measuring peak areas and comparing them with known amounts of the standard (Sigma). Retention time and sensitivity were 8.5 min and 50 pg respectively.

Experimental protocols

Experiment 1 In the first experiment, cyclic females in metoestrous were killed 15 min after administration of vehicle, NMDA (15 or 30 mg/kg) or kainic acid (2.5 or 15 mg/kg) and blood samples were collected. To determine whether the effects of NMDA and kainic acid were mediated by their specific receptors, cyclic females were killed after injection with vehicle, MK-801 (1 mg/kg) or DNQX (1 mg/kg) (at −60 min) and with vehicle, NMDA (15 mg/kg), or kainic acid (2.5 mg/kg) (at −15 min). Because the effects of different doses of NMDA and kainic acid on PRL secretion were similar, in some of the following experiments only one dose was tested.

Experiment 2 In this experiment, adult females injected on the day of birth with testosterone propionate or vehicle were ovariectomized or sham-ovariectomized. Ovariectomized females were or were not implanted with Silastic capsules (15 mm long, 1.519 mm i.d.; 3.060 mm o.d.) containing oestradiol. One week later, the animals were killed 15 min after injection of NMDA (30 mg/kg i.p.) or vehicle. Intact control females were decapitated in metoestrous. Blood samples were collected and the pituitaries removed, dissected and frozen in liquid nitrogen until required for use.
Experiment 3  Adult females injected on the day of birth with oestradiol benzoate or vehicle were ovariectomized or sham-ovariectomized and killed 1 week later, 15 min after administration of NMDA (15 or 30 mg/kg), kainic acid (2.5 or 15 mg/kg) or vehicle. Intact control females were decapitated in metoestrous.

Experiment 4  To examine whether the inhibitory effect of NMDA and kainic acid on prolactin secretion was mediated by an increase in dopamine release, cyclic and oestrogenized females were injected i.p. with vehicle or domperidone (1 mg/kg) and with vehicle, NMDA or kainic acid (15 mg/kg), 60 and 15 min, respectively, before being killed. Intact control females were decapitated in metoestrous.

Experiment 5  To find out whether NMDA and kainic acid inhibited PRL secretion in stress-induced hyperprolactinaemia, cyclic and androgenized females were decapitated 15 min after administration of NMDA (30 mg/kg), kainic acid (2.5 mg/kg) or vehicle. Before being killed, the animals were or were not exposed to ether for 5 min (23). Intact control females were decapitated in metoestrous.

Experiment 6  We have reported previously (9) that kainic acid and NMDA inhibited PRL secretion in prepubertal female rats. To determine whether the inhibitory effect at this age was related to serum PRL concentrations or endogenous dopaminergic tone at the moment of drug administration, 30-day-old female rats were injected with 250 mg/kg α-MPT or 500 mg/kg DDC 3 h before administration of vehicle or kainic acid (2.5 mg/kg) and the animals were killed 15 min later. Also, females ovariectomized or sham-ovariectomized on day 23 were killed on day 30, 15 min after administration of vehicle or kainic acid (2.5 mg/kg).

Statistics  All results are expressed as mean ± S.E.M. Data were analysed by one- or two-way analysis of variance (ANOVA) followed by Tukey’s test.

Results  Experiment 1: Effects of NMDA and kainic acid on PRL secretion in cyclic and prepubertal female rats (Table 1)  Serum prolactin concentrations in cyclic females killed at metoestrous increased significantly 15 min after administration of NMDA, the responses being similar after administration of both 15 and 30 mg/kg. The stimulatory effect of NMDA was mediated by its specific receptors and was selectively blocked by pretreatment with MK-801. Kainic acid was ineffective in adult females, but inhibited prolactin secretion in prepubertal females.

Experiment 2: Effects of NMDA on PRL secretion and pituitary dopamine concentrations in androgenized females (Figs 1 and 2)  In control females, ovariectomy significantly decreased serum PRL concentrations, which increased above the values observed in intact females 1 week after treatment with oestradiol (Fig. 1). Administration of NMDA (30 mg/kg) increased PRL secretion in intact, ovariectomized and oestrogen-treated ovariectomized control females (Fig. 1). Pituitary concentrations of dopamine remained unchanged after administration of NMDA to intact and ovariectomized females, and increased in control ovariectomized females treated with oestradiol (Fig. 1). Androgenized females showed hyperprolactinaemia (134 ± 21 ng/ml compared with 12.7 ± 4 ng/ml in controls) and pituitary concentrations of dopamine (44.1 ± 4.10 ng/g) that were similar to those in controls (35.6 ± 1.7 ng/g). Ovariectomy reduced serum PRL concentrations in androgenized females, whereas a significant increase was observed after treatment with oestradiol (Fig. 2). Despite the fact that administration of NMDA increased pituitary concentrations of dopamine in androgenized females, serum PRL concentrations remained unchanged in intact ovariectomized and oestrogen-treated ovariectomized groups (Fig. 2).

Experiment 3: Effects of NMDA and kainic acid on PRL secretion in oestrogenized females (Table 2)  Oestrogenized females exhibited hyperprolactinaemia. Serum PRL concentrations decreased significantly 1

### Table 1  Serum PRL concentrations in adult and prepubertal female rats after treatment with NMDA, kainic acid (KA), MK-801 or DNQX. Values are given as means ± S.E.M., n = 8–12 animals/group. Animals were killed 15 min after injection with NMDA or kainic acid. MK-801 and DNQX were injected 60 min before the animal was decapitated.

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>PRL (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 days</td>
<td>Vehicle</td>
<td>12.70 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>NMDA (15 mg/kg)</td>
<td>40.50 ± 11.3**</td>
</tr>
<tr>
<td></td>
<td>NMDA (30 mg/kg)</td>
<td>31.30 ± 15.2**</td>
</tr>
<tr>
<td></td>
<td>KA (2.5 mg/kg)</td>
<td>9.60 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>KA (15 mg/kg)</td>
<td>6.40 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>NMDA (15 mg/kg) + MK-801 (1 mg/kg)</td>
<td>7.40 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>NMDA (15 mg/kg) + DNQX (1 mg/kg)</td>
<td>30.50 ± 3.4**</td>
</tr>
<tr>
<td></td>
<td>KA (2.5 mg/kg) + MK-801 (1 mg/kg)</td>
<td>4.90 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>KA (2.5 mg/kg) + DNQX (1 mg/kg)</td>
<td>4.40 ± 0.6</td>
</tr>
<tr>
<td>30 days</td>
<td>Vehicle</td>
<td>20.60 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>KA (2.5 mg/kg)</td>
<td>3.90 ± 0.7**</td>
</tr>
</tbody>
</table>

** P < 0.01 compared with vehicle-injected group (ANOVA followed by Tukey’s test).
week after ovariectomy. Administration of NMDA and kainic acid significantly inhibited PRL secretion in intact and ovariectomized oestrogenized females.

**Experiment 4: Effects of combined administration of domperidone, NMDA and kainic acid on PRL secretion in control and oestrogenized females (Fig. 3)**

Domperidone injected 60 min before the animal was decapitated significantly increased serum PRL concentrations in cyclic females (198 ± 10 ng/ml compared with 9 ± 1 ng/ml in the vehicle-injected group). This response was maintained after combined administration of domperidone, NMDA and kainic acid.

**Table 2** Serum PRL concentrations in intact and ovariectomized oestrogenized females 15 min after treatment with NMDA or kainic acid (KA). Values are given as means ± S.E.M.; n = 8–12 animals/group.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intact</th>
<th>Ovariectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>115 ± 6</td>
<td>37 ± 9</td>
</tr>
<tr>
<td>NMDA (15 mg/kg)</td>
<td>38 ± 12**</td>
<td>12 ± 4**</td>
</tr>
<tr>
<td>NMDA (30 mg/kg)</td>
<td>20 ± 5**</td>
<td>NM</td>
</tr>
<tr>
<td>KA (2.5 mg/kg)</td>
<td>8 ± 1**</td>
<td>6 ± 1**</td>
</tr>
<tr>
<td>KA (15 mg/kg)</td>
<td>22 ± 6**</td>
<td>NM</td>
</tr>
</tbody>
</table>

**P** ≤ 0.01 compared with vehicle-treated group (ANOVA followed by Tukey’s test). NM, not measured.
of domperidone with NMDA or kainic acid (15 mg/kg) (Fig. 3). In oestrogenized females, serum PRL concentrations were significantly reduced after administration of NMDA and kainic acid, whereas domperidone increased serum PRL concentrations significantly and blocked the inhibitory effect of NMDA and kainic acid (Fig. 3).

Experiment 5: Effect of NMDA and kainic acid in hyperprolactinaemia induced by exposure to ether (Fig. 4)

In cyclic, non-stressed rats, serum PRL concentrations increased significantly 15 min after NMDA administration. Exposure to ether for 5 min increased serum PRL concentrations both in cyclic and in androgenized females. NMDA (30 mg/kg) and kainic acid (2.5 mg/kg) significantly decreased the serum PRL concentrations in cyclic and androgenized females exposed to ether (Fig. 4).

Experiment 6: Effect of kainic acid on hyper- and hypoprolactinaemic prepubertal female rats (Table 3)

Serum PRL concentrations decreased significantly 3 h after administration of DDC (an inhibitor of dopamine-β-hydroxylase that enhances dopaminergic tone) and increased 3 h after administration of α-MPT (an inhibitor of tyrosine hydroxylase that decreases dopaminergic tone). Kainic acid significantly reduced PRL secretion in females pretreated with vehicle, DDC or α-MPT (Table 3).

Serum PRL concentrations decreased 1 week after ovariectomy and the inhibitory effect of kainic acid was significant in both intact animals (5.1 ± 0.9 ng/ml compared with 33.8 ± 4.9 ng/ml in the vehicle-injected group) and ovariectomized animals (2.8 ± 0.5 ng/ml compared with 15.4 ng/ml in the vehicle-injected group).

Discussion

The effects of NMDA and kainic acid on serum PRL concentrations derives from their hypothalamic and pituitary actions. At the hypothalamic level, a dual role through the stimulation/inhibition of the secretion of dopamine and PRL releasing factors (PRFs) has been proposed (10, 11, 13, 25, 26). At the pituitary level, NMDA and kainic acid inhibited PRL release (13, 26).

The present results indicate that NMDA has a dual effect on prolactin secretion that is independent of pre-existing prolactin concentrations and of dopamine activity, whereas kainic acid is only inhibitory. NMDA stimulated PRL secretion in cyclic females, in agreement with previous data (6, 9, 11). The similar pituitary concentrations of dopamine after administration of vehicle or NMDA suggest that the stimulatory action of NMDA was not mediated by a decrease in the inhibitory dopaminergic tone. More probably, an increase in the release of some PRFs is involved in the NMDA-stimulated PRL release in cyclic females. Failure of NMDA to stimulate PRL secretion after blockade of dopaminergic receptors with domperidone suggests that, in the absence of dopaminergic inhibition, the stimulatory effect of PRFs were masked and further increases in serum PRL concentrations could not be detected. Alternatively, the effects of PRFs might require a certain degree of dopaminergic inhibition, as the stimulatory effects of serotonin, thyrotrophin-releasing hormone or vasoactive intestinal polypeptide on PRL release were not detected in absence of dopaminergic inhibition (27–30).

The effect of NMDA on PRL secretion is age-dependent, as NMDA inhibited prolactin release in prepubertal rats (9, 26), probably through an increase in dopamine release, as concentrations of dopamine were increased in the pituitary and decreased in the
hypothalamus after NMDA treatment. Differences in the effects of NMDA on PRL secretion in prepubertal and adult females are likely to be related to their different effects on tuberoinfundibular dopamine activity, as suggested by the different effects of NMDA on pituitary dopamine concentrations.

The hypothesis that the effects of excitatory amino acids on PRL secretion depend on serum PRL concentrations before drug administration (10, 11) is unlikely to be valid, because (a) the NMDA stimulatory effect in cyclic females remained when PRL concentrations decreased after ovariectomy or increased in response to oestradiol treatment, (b) the NMDA inhibitory effects in oestrogenized females were observed after the post-ovariectomy decrease in PRL concentrations, and (c) NMDA did not show an inhibitory effect in hyperprolactinaemia induced by neonatal androgenization. Furthermore, the inhibitory effects of kainic acid in prepubertal females was independent of prevailing PRL concentrations and was observed after treatment with \( \alpha \)-MPT or DDC and after ovariectomy.

Although it has been generally accepted that neonatal administration of testosterone permanently alters the function of the hypothalamic–pituitary axis through its aromatization to oestradiol (31), some experimental findings suggest that the aromatization theory does not explain certain differences observed in the control of prolactin secretion after neonatal administration of testosterone or oestradiol (16, 32). The present results reinforce this conjecture, as NMDA was ineffective in reducing serum PRL concentrations in androgenized, but not in oestrogenized, females.

Systemic administration of NMDA and kainic acid strongly inhibits PRL secretion in oestrogenized females, a finding previously reported for oestrogenized males (12, 13). In oestrogenized females, the endogenous dopaminergic tone seemed to be strongly reduced (33). Possibly, NMDA and kainic acid inhibited PRL secretion in oestrogenized females via increasing endogenous release of dopamine, as the effect was prevented by the administration of domperidone.

Acute stress increased PRL secretion by mechanisms involving either increased secretion of PRFs or inhibition of dopamine release (23). In cyclic and androgenized females, exposure to ether increased PRL secretion; this effect was blocked by NMDA or kainic acid treatment, which suggests that either the increased PRF secretion or the decreased dopamine release induced by stress might be counteracted by activation of NMDA and kainic acid receptors. The ability of NMDA to reduce PRL secretion in stressed androgenized females contrasts with results obtained in non-stressed androgenized females, and suggests that the pathways involved in the PRL response to stress were not affected by neonatal androgenization.

We conclude that the effects of NMDA and kainic acid on PRL secretion: (a) were not related to basal PRL concentrations, (b) probably included a change in the

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**Table 3** Serum PRL concentrations in 30-day-old females pretreated with diethyldithiocarbamate (DDC, 500 mg/kg) or \( \alpha \)-methyl-\( \beta \)-tyrosine (\( \alpha \)-MPT, 250 mg/kg), and killed 15 min after administration of kainic acid (KA, 2.5 mg/kg). Values are given as means ± S.E.M. (number of animals).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vehicle (ng/ml)</th>
<th>KA (ng/ml)</th>
</tr>
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<tbody>
<tr>
<td>Vehicle</td>
<td>20.6 ± 3.80 (8)</td>
<td>3.90 ± 0.76 (9)**</td>
</tr>
<tr>
<td>DDC</td>
<td>3.6 ± 0.04 (10)**</td>
<td>1.29 ± 0.07 (10)**</td>
</tr>
<tr>
<td>( \alpha )-MPT</td>
<td>47.4 ± 10.5 (8)**</td>
<td>29.59 ± 6.83 (8)**</td>
</tr>
</tbody>
</table>

**P ≤ 0.01 compared with corresponding vehicle-injected group (ANOVA followed by Tukey’s test).**
secretion of PRFs, as increases in pituitary concentrations of dopamine after administration of NMDA were or were not accompanied by changes in PRL secretion. (c) were influenced differently by neonatal androgenization and oestrogenization, and (d) disappeared when the action of dopamine on lactotrophs was blocked by domperidone. In addition, NMDA and kainic acid blocked the mechanisms involved in stress-induced PRL secretion.

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