Effect of neonatal treatment with monosodium glutamate on dopaminergic and L-DOPA-ergic neurons of the medial basal hypothalamus and on prolactin and MSH secretion of rats

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ABSTRACT: The effect of neonatal treatment with monosodium L-glutamate (MSG) on the dopaminergic systems of the medial basal hypothalamus has been investigated using tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AADC) immunocytochemistry. Changes in plasma levels of prolactin (PRL) and α-melanocyte-stimulating hormone (MSH) have also been determined in intact and in MSG-treated rats after inhibition of TH by α-methyl-p-tyrosine (α-MpT) or without inhibition of enzyme activity. Monosodium glutamate resulted in a 40% reduction in the number of TH immunopositive tuberoinfundibular neurons, but no change in the number of AADC-positive tuberoinfundibular nerve cells, indicating that this reduction has occurred mainly in TH-positive but AADC-negative elements, i.e., in L-DOPA-ergic neurons. In contrast, MSG did not cause changes in the number of TH and AADC immunoreactive neurons of the periventriculohypophysial and tuberohypophysial dopaminergic systems, and it did not influence basal plasma PRL levels. α-methyl-p-tyrosine has increased plasma PRL concentrations in both control and MSG-treated rats of both sexes, but significantly higher responses were detected in females. None of the treatments had any effect on plasma MSH level. These findings suggest that MSG affects primarily L-DOPA-ergic neurons located in the ventrolateral part of the arcuate nucleus, but not dopaminergic neurons situated in the dorsomedial part of the arcuate nucleus; neither PRL nor MSH secretion is altered by MSG; a significant sex difference exists in the pituitary PRL response to inhibition of TH, and this response is not affected by MSG.

KEY WORDS: Arcuate nucleus, Tyrosine hydroxylase, Aromatic L-amino acid decarboxylase, Immunocytochemistry.

INTRODUCTION

The hypothalamic dopaminergic neurons form three functionally independent and morphologically distinct systems. Neurons arising from the rostral-periventricular regions of the hypothalamus and terminating in the intermediate lobe of the pituitary gland form the periventriculohypophysial dopaminergic (PHDA) system [18–21]. These neurons are responsible for the control of α-melanocyte-stimulating hormone (MSH) secretion of the intermediate lobe [9,20,21,47,48]. The second subpopulation of neurons originates from the rostral portion of the hypothalamic arcuate nucleus dorsally adjacent to the PHDA system, and it terminates in both the neural lobe and the intermediate lobe of the pituitary gland [8]. It is called the tuberohypophysial dopaminergic (THDA) system that forms the osmosensitive hypothalamic dopaminergic system [1,25,42] and participates in the control of anterior lobe prolactin (PRL) secretion [8,14–16,34]. The third group of cells, the tuberoinfundibular dopaminergic (TIDA) system is located in the midportion and caudal portion of the arcuate nucleus and is well accepted as a major tonic regulator of the adenohypophysial PRL secretion [6,7,18,19]. Recently, it has been demonstrated that only a part of the TIDA neurons contains those two enzymes, tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AADC), which are indispensable for dopamine synthesis. It has been shown in perinatal [5] as well as in adult rats [26,30,32,35,36] that neurons located in the dorsomedial part of TIDA contain both enzymes. Thus, they may be termed as “the genuine” dopaminergic neurons, whereas those originating from the ventrolateral part of the arcuate nucleus contain only TH, but not AADC, and therefore, they can be considered as an L-DOPA-ergic neuronal system [32].
In our present studies, we have investigated the sensitivity of the rostro-caudal subdivisions of hypothalamic neuroendocrine dopaminergic neurons, i.e., PHDA, THDA, and TIDA systems and their terminal fields in the median eminence, intermediate and neural lobe of the pituitary gland, respectively, to monosodium L-glutamate (MSG) with special emphasis on the dorsomedial dopaminergic and ventrolateral L-DOPA-ergic neurons of the TIDA system. To get information about the effect of MSG, the number of TH and AADC immunoreactive neurons was counted. We have also determined the basal level of plasma PRL and MSH in MSG-treated female and male rats as well as changes in the concentrations of these hormones after inhibition of TH by α-methyl-p-tyrosine (α-MpT) in intact and in MSG-treated animals of both sexes.

**MATERIALS AND METHODS**

Newborn Sprague-Dawley rats of both sexes were injected with MSG (4 mg/g body weight subcutaneously) on days 2, 4, 6, 8, and 10 of age [36,45]. The control group of rats received NaCl in the same solution on the same days. All rats were housed in a temperature (23°C) and light-controlled (lights on from 0500 to 1900 h) room with free access to rat chow and tap water.

Two months after MSG treatment, blood samples were taken for determining plasma PRL concentrations [2], and the animals (five from each sex and from littermates without MSG treatment) were perfused under deep anesthesia with 200-ml saline, followed by 250-ml 4% paraformaldehyde via transcardiac puncture. After perfusion, the brains and the pituitary glands were removed and placed in the same fixative at 4°C for 24 h. Then they were transferred to a buffered sucrose solution (25% sucrose in PBS) for 20°C. After 10% room with free access to rat chow and tap water.

**RESULTS**

**Distribution of TH and AADC-Immunoreactive Neurons**

In the rostral periventricular region (PHDA/THDA system) of intact female rats, most of the TH immunopositive neurons exhibited also AADC-immunoreactivity, indicating that most of these neurons are dopaminergic. In the same region of intact male rats, only two-thirds of the TH positive nerve cells were also AADC immunoreactive, indicating that these neurons are dopaminergic and another one-third is L-DOPA-ergic (Table 1).

**TREATMENT**

Treatment with MSG has not caused considerable cell damage of the neurons in the rostral periventricular region (Fig. 1). The number of immunoreactive cells for both TH and AADC of this region were almost identical to those of untreated controls (Table 1). In agreement with this, no appreciable differences existed in the amount and distribution of TH or AADC immunopositive fibers at

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th></th>
<th>Male</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>MSG</td>
<td>Control</td>
<td>MSG</td>
</tr>
<tr>
<td>PHDA/THDA TH</td>
<td>63.7 ± 4.5§</td>
<td>62.0 ± 3.7</td>
<td>59.0 ± 5.4</td>
<td>61.2 ± 5.0</td>
</tr>
<tr>
<td>AADC</td>
<td>54.5 ± 1.8</td>
<td>56.2 ± 3.7</td>
<td>41.2 ± 1.1§</td>
<td>39.6 ± 1.6§</td>
</tr>
<tr>
<td>TIDA TH</td>
<td>57.8 ± 6.6</td>
<td>38.3 ± 6.8**</td>
<td>66.6 ± 5.8</td>
<td>40.5 ± 3.3**</td>
</tr>
<tr>
<td>AADC</td>
<td>26.3 ± 8.5*</td>
<td>33.8 ± 5.7</td>
<td>33.8 ± 2.6*</td>
<td>32.4 ± 2.1</td>
</tr>
</tbody>
</table>

§ Mean ± SEM obtained from five animals of both sexes of each group.
* Values of AADC-immunoreactive perikarya are significantly different (p < 0.05) from values of TH-immunoreactive neurons in the same region.
** Values are significantly different (p < 0.05) from control values.
the terminal field of these neuronal systems (in the intermediate and neural lobe of the pituitary gland) of MSG-treated rats and untreated controls (Fig. 2).

At the midlevel and posterior level of the arcuate nucleus (TIDA system) of control animals, intensely stained TH-immunoreactive cells could be detected in both the dorsomedial and ventrolateral part of the nucleus, whereas AADC-immunoreactive perikarya could be found only in the dorsomedial portion of the cell group (Fig. 3A,C). About half of the TH immunoreactive neurons were also AADC immunoreactive, and the other half was AADC-negative, indicating that half of the TIDA neurons are dopaminergic, and the other half are L-DOPA-ergic (Table 1).

Monosodium L-glutamate treatment resulted in about a 35–40% reduction of the TIDA neurons immunoreactive for TH (Table 1). The TH immunopositive neurons almost completely

FIG. 1. Photomicrographs show tyrosine hydroxylase (TH)-immunoreactive cells (A,B) and L-amino acid decarboxylase (AADC)-immunoreactive cells (C,D) in the rostral periventricular-arcuate region (representing PHDA and rostral THDA neurons) obtained from intact (A,C) and monosodium L-glutamate (MSG)-treated (B,D) male rats. V3: third ventricle. Bars represent 100 μm.
disappeared from the ventrolateral part of the cell group, without significant change in the presence of such nerve cells in the dorsomedial part of the nucleus (Fig. 3B). At the same time, at these levels of the arcuate nucleus, the number of AADC immunoreactive cells in the MSG-treated rats was very similar to the controls (Table 1, Fig. 3C,D). This indicates that the marked reduction in TH immunoreactive neurons was primarily caused by the reduction of neurons expressing TH only, which are the L-DOPA-ergic neurons.

No sexual differences existed in the morphological appearance of dopaminergic and L-DOPA-ergic neurons of the TIDA system in intact as well as in MSG-treated animals (Table 1).

**Plasma PRL and MSH Levels in MSG Treated Animals Without Additional Treatment or Receiving αMpT**

No changes occurred in basal level of plasma PRL of MSG-treated female and male animals (Fig. 4A). Inhibition of TH with α-MpT
increased plasma PRL concentrations in both sexes; the increase has been considerably higher in female than in male rats (Fig. 4A). Monosodium L-Glutamate treatment did not interfere with PRL responses to inhibition of TH (Fig. 4A).

Basal levels of plasma MSH have tended to be slightly decreased in female rats treated with MSG, but this effect was not statistically significant (Fig. 4B). α-methyl-p-tyrosine treatment did not cause significant alterations in plasma MSH levels in controls or in MSG-treated rats (Fig. 4B).

**DISCUSSION**

Our present observations, first of all, confirm previous findings showing that only the dorsomedial part of TIDA neurons produces
on demonstration that neonatal treatment of female and male rats with catecholaminergic cells [34]. Our present study confirms previous findings indicating that the majority of PHDA neurons located in the dorsomedial part of the arcuate nucleus are affected by the neurotoxic effect of MSG [12,31]. In contrast to all of these, basal levels of plasma PRL are not much different. In addition, we provide the first evidence that the majority of PHDA neurons in the intermediate lobe of the pituitary gland are resistant to the toxic effects of MSG, which is in agreement with previous findings indicating that dopamine levels in the posterior pituitary are not significantly altered by MSG treatment [12,31].

No changes occurred in plasma PRL levels of MSG-treated rats. This finding is in accordance with literary data [2,11,34]. Only one report exists [24] on increased plasma PRL levels of MSG-treated rats. Taking into account that MSG primarily affects L-DOPA-ergic neurons at the midlevel and posterior level of the arcuate nucleus and, at the same time, this treatment did not cause alterations in plasma PRL levels, it may be concluded that these L-DOPA-ergic neurons are less involved in the control of basal PRL secretion than are dopaminergic TIDA neurons.

Although, no sexual differences existed in the morphological appearance of hypothalamic L-DOPA-ergic and dopaminergic neurons of intact male and female rats, marked and significant differences could be detected in their responses after inhibition of the first essential enzymatic step involved in L-DOPA/dopamine biosynthesis. Differences in PRL responses induced by inhibition of TH between female and male rats confirm previous suspicions that biosynthetic and metabolic activity of hypophysiotrophic dopaminergic neurons that regulate pituitary PRL secretion is higher in females than in males [3,4,17,23,29,45]. It has been previously shown that the expression of TH mRNA and the enzyme activity are about threefold higher in the arcuate nuclei and stalk-median eminence, respectively [3,4], and dopamine levels in hypophysial stalk plasma are 5–7 times higher in females than in males [7,13,23]. In contrast to all of these, basal levels of plasma PRL are not much different.

It is well known that dopamine, released from terminals of PHDA neurons in the intermediate lobe of the pituitary gland [25,42], tonically inhibits the secretion of MSH from melanotropes [9,28,47,48]. Therefore, we could expect parallel changes in plasma levels of PRL and MSH during acute inhibition of TH.

**FIG. 4. Effect of α-methyl-p-tyrosine (α-MpT) (8 mg/kg, via intrajugular cannula) on plasma levels of prolactin (PRL) (A) and α-melanocyte-stimulating hormone (MSH) (B) of intact and MSG-treated female and male rats. Each value is the mean ± SEM. *p < 0.05 (plasma levels of PRL detected at 0 time vs. different time intervals after α-MpT injection). In each group, the number of rats was 7–9.**

dopamine in adult male and female rats, whereas the ventromedial part synthesizes L-DOPA as an end product [26,30,32,35,36]. In addition, we provide the first evidence that the majority of PHDA and THDA neurons express both enzymes, i.e., TH and AADC; consequently they can be considered to be "genuine" dopaminergic systems like the dorsomedial TIDA neurons.

It is well known that neonatal treatment with MSG results in a disappearance of a large number of nerve cells from the midlevel and posterior level of the hypothalamic arcuate nucleus [22,29,34,46]. A loss of several chemically identified hypophysiotrophic neurons occurs [8], but most of them have been found to be catecholaminergic cells [34]. Our present finding is the first demonstration that neonatal treatment of female and male rats with MSG primarily affects TH-positive and AADC-negative, consequently, L-DOPA-ergic, but not dopaminergic neurons of this nucleus. The relative resistance of TH- and AADC-positive neurons located in the dorsomedial part of the arcuate nucleus to MSG treatment may be caused by the maturation process of the blood brain barrier at the median eminence-arcuate nucleus region [41]. The existence of a barrier between the dorsomedial and ventrolateral parts of the arcuate nucleus is strongly supported by the finding that injection of horseradish peroxidase into the ventromedial nucleus diffuses into the adjacent dorsomedial area of the arcuate nucleus, but the ventrolateral part of it remains free of label [43]. Permeability studies of capillaries to horseradish peroxidase and immunocytochemical analyses using blood brain barrier markers also indicate that in the rat, the blood brain barrier develops during the first postnatal week [10,44]. The nature and the functional significance of this barrier during early postnatal life, however, needs to be further investigated.

Our findings clearly show that TH-positive neurons sensitive to the neurotoxic effect of glutamate are located in the ventrolateral part of the arcuate nucleus. However, this is accompanied by a shrinkage and dislocation of the dorsomedial parts toward the median eminence (Fig. 3). We could not observe significant change in the amount of TH-positive fibers of the median eminence. It should, however, be pointed out that quantitative analysis of densely innervated areas, like the median eminence, is very difficult.

Our observations confirm previous findings indicating that MSG does not affect other dopaminergic perikarya located rostrally from the TIDA neurons, namely, in the PHDA and THDA systems [31,34,46], and in agreement with this, they show that MSG does not alter MSH secretion. As far as the observed resistance of THDA neurons to the toxic effects of MSG is concerned, it is also in agreement with a previous finding that dopamine levels in the posterior pituitary are not significantly altered by MSG treatment [12,31].

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However, this was not the case. Inhibition of TH by injecting α-MpT did not increase plasma levels of MSH (Figs. 4 and 5), whereas the same dose of enzyme inhibitor induced significant increase of plasma PRL. To explain this discrepancy, one possibility could be that a difference in time exists in the response of PRL and MSH to the decrease in dopamine induced by α-MpT. Penny and Thody [40] obtained the first experimental findings in this respect. They found an increase of serum MSH after treatment with drugs, including α-MpT, which modify catecholaminergic neurotransmission. However, according to this most frequently cited paper, the increase was observed 3–4 h after injection of α-MpT. In contrast, in our experiments, we detected the level of plasma MSH during the first 60 min after inhibition of TH (Fig. 4).

An other possibility could be to assume difference in the sensitivity of the two dopaminergic systems. Difference in the sensitivity of dopaminergic regulatory systems innervating intermediate lobe (PHDA and THDA neurons) consequently regulating MSH release compared with those neurons that mainly regulate PRL secretion of anterior lobe (TIDA neurons) is not the first experimental finding of this kind. In agreement with our results, recent studies have demonstrated that D2 selective receptor antagonist (remoxipride) and agonist (quinelanore) increases [38] and reduces [17] plasma PRL levels, respectively, without altering concentrations of MSH [17,38]. Moreover, mice with a disrupted D2 dopamine receptor gene had chronic hyperprolactinemia and developed mammatrope hyperplasia, but in agreement with the above-mentioned pharmacological data, the mutant mice had no hyperplasia of the intermediate lobe [27,45].

As far as the possible function of L-DOPA neurons is concerned, they probably participate in other hypothalamic neuroendocrine regulatory mechanisms than the inhibitory regulation of PRL secretion. This population of neurons may have significance during changes in the secretory pattern of pituitary PRL, like estrous cycle or lactation. L-DOPA may be a substrate of further, and presently unknown, enzymatic processes resulting in a different hypothalamic hypophysiotrophic factor. Besides our previous and present data, this hypothesis is supported by the fact that no cycling changes occur in anterior lobe hormone secretion, including PRL, after MSG treatment of the animals [11,22]. Moreover, it has been also demonstrated that the subpopulation of cells in the arcuate nucleus that specifically bind estrogen are destroyed by neonatal treatment of MSG [22]. These hypotheses as well as other possible regulatory function of L-DOPA-ergic neurons need to be further investigated.

In summary, we presented data indicating that neonatal treatment with MSG primarily affects L-DOPA-ergic TIDA neurons without altering the number of dopaminergic nerve cells of the periventriculo-arcuate regions of the hypothalamus and basal plasma PRL levels. Further, we have demonstrated that a significant sex difference exists in the pituitary PRL response to inhibition of TH, and this response is not affected by MSG. Finally, we have provided data showing that treatment of newborn rats with MSG combined with inhibition of TH or without inhibition of enzyme activity did not influence MSH secretion in adulthood.

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