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Glutamate-Induced Serotonin Depletion in Fetal Rat Brainstem Cultures

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ABSTRACT

Exposure of dissociated cultures from fetal rat brainstem to glutamate for upto 6 h decreased cellular contents of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in a concentration- and time-dependent manner. In addition, glutamate induced lactate dehydrogenase leakage. Tetrodotoxin did not block the effects induced by glutamate. MK-801 (1 μM), an N-methyl-D-aspartate (NMDA) channel blocker, but not 6-cyano-2,3-dihydroxy-7-nitro-quinoxazoline (CNQX; 3 μM), a non-NMDA receptor antagonist, blocked glutamate-induced effects, indicating that these glutamate-induced responses are mediated through NMDA receptors.

Key Words: Serotonin depletion, Glutamate, MK-801, Brainstem culture (rat)

INTRODUCTION

L-Glutamate is the major excitatory amino acid transmitter in the vertebrate central nervous system (CNS). Recently, evidences for the role of glutamate in the regulation of central serotonin (5-hydroxytryptamine, 5-HT) neurons have accumulated. For example, raphe nuclei receives a glutamatergic input from the periaqueductal gray (Beitz, 1990; Wiklund *et al.*, 1988) and the lateral habenular nuclei (Kalen *et al.*, 1985, 1986, 1989). Application of L-glutamate to the raphe regions *in vivo* increases the firing rate of 5-HT neurons (Aghajanian *et al.*, 1972; Bramwell and Gonye, 1976), and enhances the neurotransmitter release in the terminal regions (Hery *et al.*, 1979; Lin and Shian, 1991). *In vitro*, the 5-HT uptake capacity in cultured serotonergic neurons is decreased when the cultures are exposed to high concentration of glutamate (Azmitia, 1990). In the present study, to investigate further the role of glutamate in the regulation of 5-HT cells, we studied the effect of glutamate on the cellular level of 5-HT and 5-HIAA in cultured fetal 5-HT neurons. We now report that glutamate depleted endogenous 5-HT and 5-HIAA through N-methyl-D-aspartate (NMDA) receptor.

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MATERIALS AND METHODS

Brainstem culture

Two-mm paramedian saggital strips of rhombencephali (from rhombencephalic isthmus to obex) were obtained from the brains of Sprague-Dawley rat embryos (gestation day 14) (Yamamoto *et al.*, 1981). After mechanical dissociation of the tissue, cells were plated at an approximate

density of 6×10^5 cells/well on 48-well plates previously coated with poly-L-lysine (50 μg/ml), in Dulbecco's modified Eagle's medium (DMEM) with 10% v/v heat-inactivated fetal calf serum. At 2nd day *in vitro*, cultures were shifted into DMEM containing N2 media (Bottenstein and Sato, 1979). Non-neuronal cell division was halted by 2-day exposure to 10 μM cytosine arabinoside from 2 to 4 days *in vitro*. Cultures were maintained in a humid 5% CO_2 incubator (37℃) for 7 days. At 7th day *in vitro*, cells were rinsed twice with DMEM, and exposed to 1 mM glutamate for 6 h in 0.5 ml of DMEM at 37℃ in a humid 5% CO_2 indubator. After exposure to glutamate, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels in cells and the levels of lactate dehydrogenase in the media (Vassault, 1983) were determined.

Assay of 5-HT and 5-HIAA

5-HT and 5-HIAA levels were determined by high-performance liquid chromatography with electrochemical detection (Saller and Salama, 1984). After exposure to glutamate, cultures were rinsed twice with 4℃ phosphate-buffered saline. Cells in each well were scraped and sonicated in 90 μl of 0.1 M perchloric acid. After centrifuging the homogenate and filtering the supernatant through a 0.45 μm Millipore HV-4 filter unit, 25 μl of the filtrate was injected onto C_{18} μ-Bondapak column (Waters). As a mobile phase, 0.5 M NaH_2PO_4 (adjusted to pH 3.7) containing sodium octanesulfonic acid (1 mM), disodium EDTA (0.5 mM) and acetonitrile (10% v/v) was used. The flow-rate was 1 ml/min and the oxidation potential was 0.55 V. Data were presented as means±S.E. and expressed as percent control. For comparison of two or more means Student's *t*-test or Dunnett's test was used, respectively.

RESULTS

Cellular content of 5-HT and 5-HIAA in the cultures maintained for seven days *in vitro* were 3.62±0.35 pmol/well (5.38±0.53 pmol/mg protein), and 2.28±0.34 pmol/well (3.39±0.46 pmol/mg protein), respectively. The ratio of 5-HIAA/5-HT was 0.63±0.03. Exposure of the cultures maintained for seven days *in vitro* to glutamate (0.1~1 mM) in DMEM for one to six hours decreased cellular 5-HT and 5-HIAA in a time- and concentration-dependent manner (Fig. 1a, b). Exposure of the cultures to 1 mM glutamate in DMEM for six hours decreased cellular 5-HT and 5-HIAA, by 45.2% and 48.7%, respectively (Fig. 1). The observation of change in cellular content of 5-HIAA after glutamate content was similar to that of 5-HT, without altering the ratio of 5-HIAA/5-HT.

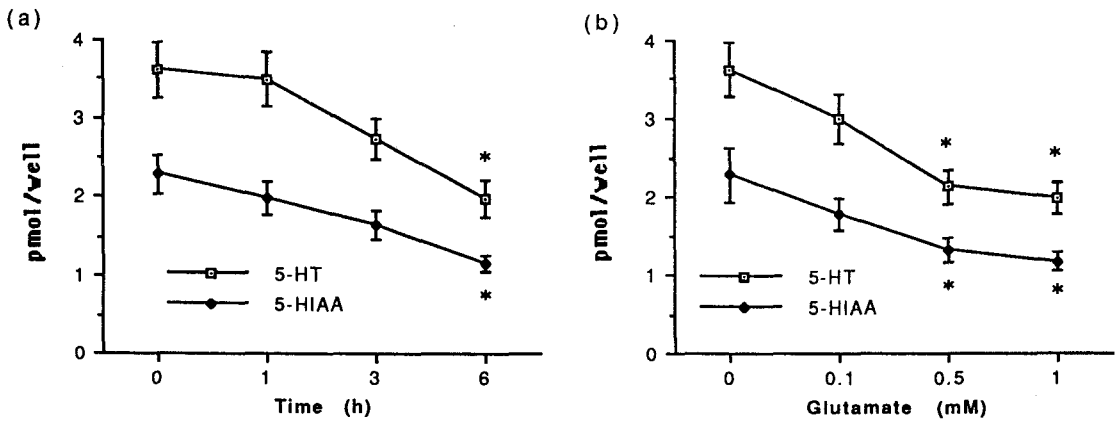

Fig. 1. Depletion of 5-HT as a function of the incubation time (a) and of concentration of glutamate (b). Seven-day old cultures were incubated for various incubation time with 1 mM glutamate (a) or for 6 h with various concentrations of glutamate (b). Values are mean±S.E. of 4 experiments. *$p<0.05$ (compared to the controls).

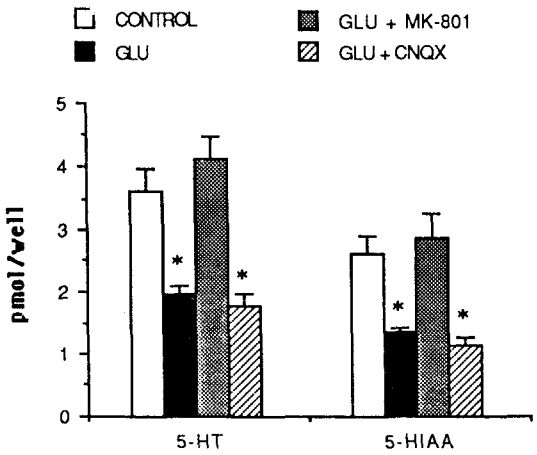

Fig. 2. Effect of glutamate antagonists on the glutamate-induced depletion of 5-HT and 5-HIAA. Seven-day old cultures were exposed to 1 mM glutamate for 6 h with or without antagonists, MK-801 [1 μM] and CNQX [3 μM]. Values are mean±S.E. of 3 experiments. *$p<0.05$ (compared to the controls). Glu; glutamate.

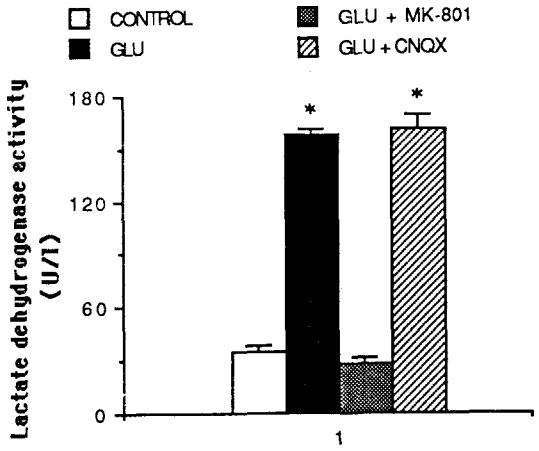

Fig. 3. Effect of glutamate antagonists on the glutamate-induced release of lactate dehydrogenase release. Seven-day old cultures were exposed to 1 mM glutamate for 6 h with or without antagonists, MK-801 [1 μM] and CNQX [3 μM]. Values are mean±S.E. of 3 experiments. *$p<0.001$ (compared to the controls). Glu; glutamate.

To determine the role of propagated action potentials in the glutamate-induced depletion of 5-HT, cultures were exposed to glutamate during blockade of voltage-dependent Na^+ channels by tetrodotoxin (TTX) (1 μM). Six hour-incubation with TTX alone did not affect cellular 5-HT content (data not shown). TTX did not affect glutamate-induced responses (data not shown).

To determine the subtype (s) of glutamate receptors involved in the glutamate-induced depletion of 5-HT, cultures were exposed to glutamate in the presence of MK-801, an NMDA channel blocker or CNQX, a non-NMDA receptor antagonist. The 5-HT depletion induced by glutamate was completely blocked in the presence of 1 μM MK-801, but not 3 μM CNQX (Fig. 2).

As glutamate can be toxic to some neurons under certain conditions, we measured activities of lactate dehydrogenase, a cytosolic marker, in the media after glutamate exposure. The incubation with glutamate for 6 hours markedly increased lactate dehydrogenase release into the media (Fig. 3). The lactate dehydrogenase release induced by glutamate was inhibited with the treatment of 1 μM MK-801, but not 3 μM CNQX.

DISCUSSION

The present study suggests that NMDA receptors on serotonin neurons are competent to deplete 5-HT as early as 7 days *in vitro*. The lack of effect of CNQX suggests that non NMDA receptors are not involved in glutamate-induced 5-HT depletion at this stage of development. This NMDA receptor-mediated 5-HT depletion is in line with previous reports on the effect of glutamate of 5-HT neurons through NMDA receptors; MK-801 prevented glutamate-induced suppression in 5-HT neuronal development in culture (Azmitia, 1990); glutamate-elicited synaptic potentials in rat dorsal raphe neurons were blocked by DL-2-amino-5-phosphonovaleric acid (APV), an NMDA antagonist (Pan and Williams, 1989). Cultures exposed to glutamate showed evidence of cytotoxicity of the general cell population, reflected by lactate dehydrogenase release. This glutamate-induced lactate dehydrogenase release was also mediated by the stimulation of NMDA re-

ceptors since it was blocked by MK-801 (Choi *et al.*, 1988).

When we used 5 day-old cultures, the 5-HT depleting effect of glutamate was markedly lessened, while in 9 day-old cultures, the effect of glutamate was more marked, suggesting the ontogenetic increase in the susceptibility to glutamate (unpublished observation). The cellular contents of 5-HIAA seems to be closely dependent on the 5-HT content in the cultured 5-HT neurons of this study, as it was changed similarly with 5-HT, with the ratio of 5-HIAA/5-HT unchanged. In summary, this report provided the evidence of glutamate modulation of serotonergic neurons through NMDA receptors in culture as early as 7 days *in vitro*.

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=국문초록=

흰쥐태 뇌간의 배양에서 Glutamate에 의한 Serotonin의 고갈

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흰쥐태 뇌간의 세포를 배양하여 glutamate에 6시간까지 노출시 glutamate의 농도 및 노출시간에 의존적으로 세포내 5-HT 및 5-HIAA의 함량이 감소하였고, 배양액으로 LDH의 유출이 증가하였다. Tetrodotoxin은 glutamate의 작용을 차단하지 못하였다. NMDA 수용체 통로 봉쇄제인 MK-801에 의해 glutamate의 작용이 효과적으로 차단되었고, non-NMDA 길항제인 CNQX는 효과가 없었으므로, serotonin 신경세포에 대한 glutamate의 작용은 NMDA 수용체의 자극에 의한 것으로 사료된다.