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Research Report

Effects of stimulation of glutamate receptors in medial septum on some immune responses in rats

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ABSTRACT

The immunomodulatory role of medial septum (MS) has been explored so far only in MS lesioned rats. But in MS lesioned rats, all the nerve cells and fibres of the lesioned area are damaged and the specific role of the neural circuits of MS on immunomodulation cannot be assessed from the lesion of MS. Considering the presence of a large number of glutamate receptors in MS, the specific role of glutamate receptors stimulation on some immune responses has been investigated in the present study. Hyperreactive behaviour, TC and DC of WBC, phagocytic activity of peripheral leukocytes, adhesibility and cytotoxicity of splenic mononuclear cells (MNC), delayed type of hypersensitivity (DTH) responses and the serum corticosterone (CORT) were measured after microinfusion of glutamate into MS of rats. To ascertain the specific role of those glutamate receptors, the parameters were also measured after microinfusion of glutamate receptor blocker 6, 7-dinitroquinoxaline-2, 3-dione (DNQX). The hyperreactive behaviour, TC and DC of WBC remained unaltered after stimulation or blocking of glutamate receptors. The phagocytic activity, adhesibility and cytotoxicity of splenic MNC, and DTH responses were increased after infusion of 0.25 and 0.5 μM glutamate. But after infusion of higher dose of glutamate (1 μM), the phagocytic activity and the adhesibility of splenic MNC were decreased and other parameters remained unaltered in that condition. After infusion of 4 and 8 mM DNQX all the observed immunological parameters were decreased. The CORT concentration was decreased after infusion of 0.25 and 0.5 μM of glutamate but it was increased after infusion of 1 μM glutamate or 4 and 8 mM DNQX. Results indicate that the medial septal glutamate receptors play an important role in the modulation of some immune responses.

Abbreviations: MS, medial septum; TC, total count; DC, differential count; MNC, mononuclear cell; DTH, delayed type of hypersensitive; DNQX-6,7, dinitroquinoxaline-2, 3-dione; CORT, corticosterone; μM, micromolar; mM, milimolar; PI, phagocytic index; BSA, bovine serum albumin; NKCC, natural killer cell cytotoxicity; C, control; CV, vehicle infused control; GLU1, 0.25 μM glutamate infused rats; GLU2, 0.5 μM glutamate infused rats; GLU3, 1 μM glutamate infused rats; DNQX1, 2 mM DNQX infused rats; DNQX2, 4 mM DNQX infused rats; DNQX3, 8 mM DNQX infused rats; PBS, phosphate buffer saline; FACS, fluorescence activated cell sorter; EDTA, ethylenediaminetetraacetic acid; FITC, fluorescein isothiocyanate; LAI, leukocyte adhesive inhibition index; LDH, lactate dehydrogenize

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1. Introduction

With the advent of neuroimmunology, the link between nervous and immune systems has been investigated by several authors to understand the process of neural regulation of immune system (Wrona, 2006; Wrona et al., 1994). Different regions of the brain were manipulated by lesion or stimulation methods to delineate its specific role on immune parameters (Cross et al., 1982; Dutta et al., 2011; Mori et al., 2000, 1993; Roszman et al., 1982; Take et al., 1995). As an autonomic and neuroendocrine controlling area of brain, the role of hypothalamus has been emphasized on immunomodulation and several investigators have explored the regulatory mechanism of hypothalamus on immune system (Tsuboi et al., 2001; Wrona, 2006; Wrona et al., 1994). Besides hypothalamus, different areas of limbic system have attracted the investigators for studying their role on immunomodulation, probably because of their association with emotional state and autonomic activity. Among the nodal areas of limbic system such as amygdala, hippocampus, septum and bed nucleus of stria terminalis were investigated in this regard (Dutta et al., 2011; Ghoshal et al., 1998; Nance et al., 1987; Wrona, 2006).

As a nodal point of limbic system medial septum (MS) has the potentials of controlling the behaviours (Dutta and Ghosh, 2011; Myhrer, 1989; Srividya et al., 2005). The locomotor and exploratory behaviours were decreased and the hyperreactive behaviour remained unaltered in rats after an electrolytic lesion of MS (Dutta et al., 2011; Myhrer, 1989; Srividya et al., 2005). Besides controlling the behaviours, MS has some immunomodulatory role (Dutta et al., 2011; Wrona, 2006). The lesion induced immunomodulatory role of MS has been reported by several authors (Dutta et al., 2011; Jurkowski et al., 2001; Labeur et al., 1991; Zach et al., 1999), but the lesion studies have some limitations as all the nerve cells and fibres of the lesioned area are damaged. So, the specific role of neuronal circuit and neurotransmitter receptors of MS on the immune changes cannot be assessed after lesion of MS.

Within MS there is a complex interneuronal circuit of cholinergic, GABAergic and glutamatergic neurones (Gritti et al., 1997; Kiss et al., 1997; Manseau et al., 2005). The cholinergic, GABAergic and glutamatergic neurones of MS receive AMPA receptor mediated synaptic input from local glutamatergic neurons of MS (Manseau et al., 2005). These local glutamatergic neurons of MS can generate powerful excitatory influences to glutamatergic, cholinergic and GABAergic neurons of MS. With these complex interneuronal circuits, MS receive huge afferent fibres from hypothalamus, olfactory bulb, hippocampus and amygdala Swanson and Crown (1979). It sends efferent fibres to entorhinal, cingulate, medial prefrontal, olfactory and insular cortex, hypothalamus, hippocampus, midbrain, dorsal and ventral raphe (Swanson and Crown, 1979).

In the present study, the glutamate receptors of MS were stimulated to assess the specific role of these receptors on some immune parameters e.g. total count (TC) and differential count (DC) of WBC, phagocytic activity of peripheral leukocytes, adhesibility of splenic mononuclear cell (MNC), cytotoxicity of splenic MNC and delayed type of hypersensitivity (DTH) responses in rats. These immune responses were found to be altered in MS lesioned rats (Dutta et al., 2011). For the stimulation of medial septal glutamate receptors, the putative neurotransmitter glutamate was microinfused into MS in rats. To ascertain the specific effects of glutamate induced stimulation of MS on the immunological parameters, the glutamate receptor blocker 6, 7-dinitroquinoxaline-2, 3-dione (DNQX) was microinfused into the MS in separate experiments. In addition to immunological parameters the hyperreactive behaviour and serum corticosterone (CORT) concentration were measured after microinfusion of glutamate or DNQX into MS in rats.

2. Results

2.1. *Experiment I*

After microinfusion of glutamate into MS, the mean hyperreactivity scores of GLU1 (0.25 μM glutamate), GLU2 (0.50 μM glutamate) and GLU3 (1.00 μM glutamate) rats remained unaltered compared to that of C and CV rats. The mean hyperreactivity scores of DNQX1 (2 mM), DNQX2 (4 mM) and DNQX3 (8 mM) rats also remained unaltered compared to that of C and CV rats (Table 1).

2.2. *Experiment II*

2.2.1. *TC and DC of WBC*

The TC of WBC was not significantly changed in GLU1, GLU2 and GLU3 rats compared to that of C and CV rats (Table 2). The TC of WBC also remained unaltered in DNQX1, DNQX2 and DNQX3 rats compared to that of C and CV rats (Table 2). The percentage of neutrophils, lymphocytes and monocytes was not changed after microinfusion of any doses of glutamate or DNQX compared to that of C and CV rats.

2.2.2. *Phagocytic activity of peripheral leukocytes*

The phagocytic index (PI) of the GLU1 and GLU2 rats was significantly decreased compared to that of C [F (7, 40)= 19.823, $p<0.001$] and CV rats [F (7, 40)=19.823, $p<0.01$].

Table1 – The hyperreactivity scores in control, vehicle, glutamate and DNQX infused rats. The reactivity was tested on the 19th day of cannula implantation.

Groups	Hyperreactivity scores
C	0.93±0.15
CV	1.15±0.25
GLU1	1.54±0.31
GLU2	1.60±0.18
GLU3	1.49±0.18
DNQX1	1.82±0.14
DNQX2	2.04±0.42
DNQX3	1.54±0.22

Values are expressed as mean±SEM (n=6 in each group).