Role of excitatory amino acids on somatostatin production in the central nervous system

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There is increasing evidence that neuroexcitatory amino acids (in particular those related to glutamate) have a cytotoxic action on central nervous system (CNS) neurons both in vivo and in vitro and this may play a mediating role in the pathogenesis of neurological degenerative diseases such as Alzheimer’s (AD) and motor neuron disease (MND). In patients with MND, for example, there are raised levels of glutamate, aspartate and glycine in the cerebrospinal fluid [1]. This may be due to an increased release from damaged spinal cord and brain cells resulting in reduced amino acid levels in these areas [2]. Furthermore, in a form of MND occurring in S.E. Asia there is evidence for the involvement of β-N-methylamino-l-alanine, a dietary component which has agonist action at the N-methyl-D-aspartate (NMDA) receptor [3].

Abbreviations used: AD, Alzheimer’s disease; MND, motor neuron disease; NMDA, N-methyl-D-aspartate; SRIF, somatostatin; GABA, γ-aminobutyric acid; D-AP5, 2-amino-5-phosphonovaleric acid.

In patients with AD there is evidence of a reduced cortical glutamate concentration [4], together with an impairment in glutamate transmission, as judged by reduced receptor binding for NMDA and quisqualate in the hippocampal region [5]. The putative involvement of excitatory amino acids in AD has also been demonstrated in animal studies. Ibotoic acid (an NMDA agonist)-induced lesions of the nucleus basalis of Meynert in rabbits results in neuronal atrophy, the formation of plaques and tangles and a reduction in cholinergic input to the cortex [6]. Similar studies in rats have shown that γ-aminobutyric acid (GABA) neuron depletion, which is characteristic of Huntington’s chorea, can be mimicked by striatal lesioning with quinolinic acid [7].

Thus it would appear that amino acid neurotransmitters, in particular those related to activation of glutamate receptor subtypes, may play a causative role in neurodegeneration.

In this study, a rat cerebellar culture system has been used to investigate the action of excitatory and inhibitory amino acids on somatostatin (SRIF) activity (SRIF is severely depleted in the brains of AD patients) [8, 9]. Fetal rat (17 day) cortical and hypothalamic cultures were prepared as previously described and plated at a seeding density of 5 × 10^5 cells/cm^2 into 24-well tissue culture dishes pre-treated with poly-l-lysine (100 μg/ml) [10]. After an initial incubation (16 h) in α-minimal essential medium containing...
The agonists were incubated for the times indicated; values are different experiments.

Table I.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Basal</th>
<th>NMDA</th>
<th>KA</th>
<th>QUIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>107 ± 19</td>
<td>131 ± 12</td>
<td>99 ± 12</td>
<td>120 ± 21</td>
</tr>
<tr>
<td>4</td>
<td>126 ± 8</td>
<td>130 ± 4</td>
<td>87 ± 10</td>
<td>110 ± 18</td>
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<tr>
<td>10</td>
<td>231 ± 26</td>
<td>206 ± 8</td>
<td>98 ± 7</td>
<td>170 ± 19</td>
</tr>
<tr>
<td>24</td>
<td>376 ± 30</td>
<td>327 ± 20</td>
<td>124 ± 11</td>
<td>190 ± 20</td>
</tr>
<tr>
<td>48</td>
<td>267 ± 8</td>
<td>385 ± 20</td>
<td>142 ± 19</td>
<td>165 ± 21</td>
</tr>
<tr>
<td>96</td>
<td>273 ± 6</td>
<td>539 ± 45</td>
<td>152 ± 8</td>
<td>140 ± 16</td>
</tr>
</tbody>
</table>

The agonists were incubated for the times indicated; values are means ± S.E.M. (n = 4) and are representative of three to four different experiments.
bi-ephasic action was seen with kainic acid; a concentration of 10^{-5} M stimulated intracellular levels (150–200% of basal), while a 10-fold higher concentration reduced levels to 20–50% of basal.

Of the two major inhibitory amino acids in the CNS, glycine (10^{-5} M) stimulated intracellular SRIF (225–300% of basal); secreted levels were, however, not significantly altered. GABA which can be converted endogenously from glutamate in neuronal axons, did not affect either intracellular or secreted SRIF.

Preliminary experiments indicated that NMDA stimulation of SRIF release could not be inhibited by pretreatment and co-incubation with competitive antagonist, 2-amino-5-phosphonopentanoic acid (D-AP5), but subsequent experiments using a higher dose (5 x 10^{-4} M) of D-AP5 did cause a 20–30% reduction in the level of NMDA stimulation. Similar experiments with the non-competitive antagonist MK801 inhibited NMDA-stimulated SRIF release (1 x 10^{-5} M-NMDA, 150–200% of basal; 10^{-5} M-NMDA+10^{-4} M-MK801, 100–120% of basal). This latter finding, together with the fact that glycine can stimulate intracellular SRIF, suggests that the stimulatory action of NMDA on SRIF release may, at least in part, be mediated through the glycine allosteric regulatory site of the NMDA receptor. As expected neither D-AP5 nor MK801 prevented the inhibitory action of kainic acid on SRIF levels; surprisingly, however, the selective kainic acid/quisqualate antagonist, 3-glutamyltaurine also had no effect. This latter result is consistent with that reported previously [13].

A number of reports [14, 15] have suggested that a putative action of excitatory amino acids is mediation through the inositol phospholipid pathway. Our results show that addition of either glutamate or quisqualate to cortical or hypothalamic cells prelabelled with [3H]inositol caused a rapid accumulation of labelled inositol phosphates (glutamate, 180±10% of basal; quisqualate, 165±10% of basal). Kainic acid was much less potent (125±5% of basal) and NMDA showed virtually no activity. These results are similar to those obtained by Schoepf [14]. The lack of effect of NMDA on inositol phospholipid turnover may be due to the presence of Mg^{2+} in our incubating media. Mg^{2+} has been shown to inhibit NMDA, but not kainic acid or quisqualate potentiation of inositol phospholipid turnover [15]. Whether excitatory amino acid stimulation or inhibition of SRIF activity is mediated through the inositol phospholipid pathway is unclear. Our initial experiments with staurosporine (a potent protein kinase C inhibitor) [16] failed to reduce the stimulatory action of NMDA or the inhibitory action of kainic acid on SRIF release.

In summary, our data show that rat cortical and hypothalamic SRIF can be regulated by activation of glutamate and glycine receptors. The mechanisms of action of the functional link between amino acids and SRIF remains to be determined. Other reports have suggested that excitatory amino acids can inhibit stimulated inositol phospholipid turnover [17], and NMDA, but not kainic acid or quisqualate, can activate the arachidonic acid cascade system [18]. Nevertheless, the biphasic action of NMDA and kainic acid on SRIF levels is consistent with the hypothesis that excitatory amino acids under normal circumstances play a transmitter role, but have a neurotoxic action when they are present in high concentrations.

The putative accumulation of excitatory amino acids in neurodegenerative diseases may occur as a result of damage to the neuron support cells, the glial astrocytes. The primary cause of this damage is, however, unclear. Since the astrocytes are major sites of glutamate metabolism, their impairment may lead to the accumulation of glutamate and harmful ammonia and a loss of important neurotropic factors, thus ultimately leading to neurotoxicity (for a review, see [19]). Furthermore, there is evidence to suggest the presence of neurotropic molecules in sera of patients with Huntington's chorea [20] and in MND (M.D. Lewis, personal communication). Although the natures of such molecules are as yet undetermined, their toxicity may well be mediated through actions at excitatory amino acid receptors. Finally, investigations into antagonists which may reverse the excitatory amino acid action of SRIF may provide a basis for new rational therapies in neurological diseases, in particular AD.