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Destruction of Specific Hippocampal Cell Fields Increases Ornithine Decarboxylase Activity: Modulation of the Biochemical but Not the Histological Changes by Ganglioside GM1

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INTRODUCTION

Ornithine decarboxylase (ODC) is the rate-limiting enzyme in the biosynthesis of the polyamines putrescine, spermine, and spermidine (4,9,33). This enzyme is widely distributed in many types of mammalian tissue and is particularly prevalent in rapidly growing tissue such as neoplasia and in embryos. There is also evidence that changes in ODC activity reflect maturational processes occurring in the developing nervous system (31). The activity of this enzyme can be modulated by a variety of extrinsic factors. For example, ODC activity is increased several-fold following the administration of various drugs (6,14,16,28), hormones (15,29), environmental toxicants (7), and growth factors (24). ODC activity is also very responsive to neural insult with the degree of increase being related to the extent of damage (16). On the basis of these observations, it has been postulated that alterations in ODC activity reflect an involvement of polyamines in growth and differentiation of cells and in their response to injury (19,30,33).

Several lines of evidence indicate that neurotrophic factors, in particular, ganglioside GM1 (1-3) and nerve growth factor (24), exert some of their actions through an interaction with polyamines. Recently, Agnati and coworkers (1-3) have shown that ganglioside GM1 facilitates recovery of dopaminergic parameters in the corpus striatum following hemitransection of the nigrostriatal pathway and that this effect is dependent upon the permissive action of putrescine. For example, GM1's trophic effects in this model system can be attenuated by the administration of difluoromethylornithine a specific inhibitor of ODC (25). The GM1 effect can then be reinstated by direct administration of putrescine. These data indicate that polyamine synthesis, as directed by ODC, contributes in an essential way to the neurotrophic effects of GM1 ganglioside.

The following experiments examined the time-dependent effects of unilateral hippocampal damage induced by microinjection of selective cytotoxic compounds on ODC activity in the ipsi- and contralateral hippocampus (HPC). Intrahippocampal injection of kainic acid (KA) was used to destroy pyramidal neurons in area CA3, while the granule cells in the dentate gyrus were destroyed by intradentate injection of colchicine (COL). Second, we examined whether administration of GM1 ganglioside prior to surgery would alter the response of ODC to hippocampal damage on either the injected or noninjected side. Finally, we determined whether pre-treatment with GM1 would alter the profile or magnitude of hippocampal damage induced by KA or COL. Previous studies in our laboratory have shown that GM1 prevents the development of certain behavioral effects and facilitates recovery of locomotor activity and cogni-
tive function following intradentate colchicine without minimizing its effect on either the target population of neurons, dentate granule cells, or neurons which are afferent to it (18, 36, 37). These studies are part of an ongoing effort to delineate the mechanisms responsible for neural and behavioral plasticity using the HPC as a model system (34, 35).

METHODS

Subjects

Adult male Fisher 344 rats, obtained from Charles River Breeders (Wilmington, DE) were used. The animals were housed in groups of four in a temperature-controlled colony room. The colony was maintained on a 12-h light/dark cycle with lights on at 0700 h, and both laboratory chow (NIH diet 31) and tap water were continuously available. All animals were 90–120 days of age and weighed between 250 and 300 grams at the beginning of the study.

Intrahippocampal Administration of Cytotoxins

Rats were anesthetized with sodium pentobarbital (45 mg/kg) and positioned in a Kopf stereotaxic instrument. Stereotaxic surgery was performed according to a previously described protocol (37). Rats were slowly infused (0.125 µl/min) into the HPC with either artificial cerebrospinal fluid (CSF) or either 3.5 µg of COL or 0.2 µg of KA dissolved in CSF. A total volume of 0.5 µl was delivered per site. COL was injected into the left dentate gyrus at the following coordinates: 1.8 mm lateral to the sagittal suture, 2.8 mm posterior to bregma, and 3.3 mm below the cortical surface. KA was injected in the CA3 region of the HPC at the following coordinates: 4.0 mm lateral to the sagittal suture, 2.8 mm posterior to bregma, and 3.3 mm below the cortical surface. These doses of COL and KA and the injection parameters have been shown to produce an extensive loss of granule cells in the dentate gyrus and pyramidal neurons in the CA3 cell field (18, 37).

Ornithine Decarboxylase Activity

Rats were unilaterally injected with 3.5 µg of COL, 0.2 µg of KA or CSF and then sacrificed by decapitation 6, 12, 72, 210, or 960 h later. Brains were rapidly removed and placed on ice, and the HPC was dissected free. ODC was assayed by the measurement of evolved 14CO2 from carboxyl-14C-ornithine (65.9 mCi/mmol, New England Nuclear, Boston, MA) according to Russell and Snyder (30). Hippocampal tissue was homogenized in 19 vol of 0.04 M Tris–HCl buffer (pH 7.4) and the resulting homogenate was centrifuged at 26,000g for 10 min. Nine hundred microliters of the supernatant was added to 50 µl of a pyridoxyl phosphate solution (0.001 M) and 50 µl of carboxyl-14C-ornithine in 0.0445 M dithiothreitol. The final ornithine concentration was 2–5 µM.

Incubation was for 30 min at 37°C in a sealed tube in a shaking water bath and labeled CO2 was trapped on a paper wick containing hyamine, suspended above the reaction mixture. The reaction was stopped and CO2 was released by the injection of 0.5 ml 10% trichloroacetic acid into the mixture. The decarboxylation reaction was linear with time under these conditions. Background decarboxylation, not attributable to ODC, was determined by running a parallel incubation in the presence of 5 mM difluoromethylornithine, a specific inhibitor of ODC (25).

Administration of Ganglioside GM1

In the second and third experiments rats were injected intraperitoneally for 3 consecutive days with either 0.9% saline or 30 mg/kg of ganglioside GM1 prior to intrahippocampal injection of KA or CSF into CA3 or COL or CSF into the dentate gyrus. Rats were then sacrificed 24 h following surgery for analysis of ODC activity or 21 days following surgery for morphometric assessment of the HPC.

RESULTS

Increases in ODC Activity following Intrahippocampal Colchicine or Kainic Acid

Intradentate injections of COL and intrahippocampal injections of KA into the CA3 cell fields produced increases in ODC activity on both the injected and noninjected sides. The contralateral side exhibited changes which were smaller in magnitude and shorter in duration. However, the KA group exhibited a more immediate and pronounced response on the contralateral side. CSF injected into either of the hippocampal sites produced increases in ODC activity which were smaller and more transient in nature than those produced by the cytotoxins. The time course of these changes depended on the cytotoxin and the site of injection. These effects are described in detail below.

Intradentate injection of CSF produced a significant increase in ODC activity on the ipsilateral and contralateral sides when compared to nontreated hippocampi (Fig. 1). Pairwise comparisons revealed that ODC activity was elevated on both the injected and noninjected sides at 6, but not at 12, 72 or 216, hr following injection.

Intradentate COL produced a large and persistent increase in the activity of ODC on the injected side that peaked at 12 h and did not recover until 960 h after surgery (see Fig. 2 for post hoc comparisons). A 2 × 5 factorial ANOVA (as described above) revealed significant treatment (F(1,71) = 12.62), time (F(4,71) = 10.13), and treatment by time (F(4,71) = 4.16) effects, all P’s < 0.01, comparing the injected to the noninjected side.
The magnitude of the increase in ODC activity was significantly different between the COL and CSF-injected groups on both the injected and noninjected sides. Post hoc comparisons revealed that the COL-induced elevation of ODC activity was significantly larger than that produced by CSF on the injected side at all times following surgery. In contrast, the contralateral increase in ODC was larger for the COL group only at 6 and 12 h following surgery. A 2 × 5 factorial ANOVA revealed significant treatment (F's(1,66) = 8.27–13.47) and time (F's(4,66) = 3.88–9.14, all P's < 0.01) effects for both the injected and noninjected sides. Furthermore, there was a significant treatment by time interaction for the injected (F(4,66) = 4.56, P < 0.01), but not the noninjected, side (F(4,66) = 2.12, P > 0.05).

The increase in ODC activity following injection of CSF into CA3 was comparable in magnitude and time course to that observed following intradentate CSF and is not further described.

Intrahippocampal KA increased ODC activity significantly more on the injected than on the noninjected side. A 2 × 4 factorial ANOVA revealed significant treatment (F(1,54) = 3.72), time (F(3,54) = 20.64), and treatment × time (F(3,54) = 10.38) effects (all P's < 0.05). Post hoc comparisons revealed that ODC activity was significantly increased on the injected side 6, 12, and 72, but not 216, h after injection.

Comparison of ODC activity on the injected side in the KA and the CSF groups revealed significant treatment (F(1,56) = 36.38) and time (F(3,56) = 3.76) effects (P's < 0.01) but an insignificant treatment × time (F(3,56) = 1.09, P > 0.10) interaction. Post hoc comparisons demonstrated that ODC activity was significantly elevated in the KA group 12, 72, and 216 h following surgery. The lack of a significant interaction term indicates that the changes in ODC activity at the different time points were comparable for the KA and CSF groups.

KA produced an immediate and very large increase in ODC activity on the contralateral side. A factorial ANOVA comparing noninjected sides in the KA and CSF groups revealed significant treatment (F(1,58) = 50.28) time (F(3,58) = 24.39), and treatment × time (F(3,58)
Ganglioside GM1 Modulates the Increases in ODC Activity Produced by Intrahippocampal Colchicine or Kainic Acid

In the second experiment rats were injected intraperitoneally for 3 consecutive days with either 0.9% saline or 30 mg/kg of ganglioside GM1 prior to intrahippocampal injection of KA or CSF into CA3 or COL or CSF into the dentate gyrus. Surgery and dosages of the cytotoxins were according to the previously described protocol. All animals were sacrificed by decapitation 24 h following surgery. This time point was chosen since extrapolation from the time courses presented in Figs. 2 and 3 revealed that 24 h following injection of either KA or COL comparable increases (700–750% of controls) in ODC activity were evident on the injected sides.

Administration of GM1 ganglioside prior to injection of CSF into either hippocampal site did not alter the increase in ODC activity on either the injected or the noninjected side. Therefore, the CSF–SAL and GM1–CSF groups were combined to form one control group designated CON.

Ganglioside GM1 altered the magnitude and topographic pattern of ODC changes following intradentate injection of COL. One-way ANOVAs revealed significant overall treatment effects for both the injected (F(2,22) = 6.32, P < 0.01) and the noninjected (F(2,22) = 8.10, P < 0.01) sides. Post hoc analyses demonstrated that the COL–CSF group exhibited significantly increased ODC activity on the injected, but not the noninjected, side compared to the CON group. Pretreatment with GM1 prevented the increase in ODC activity on the injected side but significantly increased it on the noninjected side (Fig. 4).

The ganglioside regimen also altered the magnitude of the ODC increase induced by KA on the injected side. One-way ANOVAs revealed significant overall treatment effects for the injected (F(2,33) = 26.76, P < 0.01) and the noninjected (F(2,32) = 30.74, P < 0.01) sides. Post hoc comparisons demonstrated that KA significantly increased ODC activity on both sides. GM1 pretreatment significantly enhanced the ODC response on the injected side without affecting the contralateral response (Fig. 5).

Ganglioside GM1 Does Not Alter the Hippocampal Damage Induced by Colchicine or Kainic Acid

In the final experiment rats were injected intraperitoneally for 3 consecutive days with either 0.9% saline or 30 mg/kg of ganglioside GM1 prior to the unilateral injection of KA or CSF into CA3 or COL or CSF into the dentate gyrus. Surgery and dosages of the cytotoxins were according to the previously described protocol. All animals were sacrificed 21 days following surgery.

Anesthetized animals were transcardially perfused by a peristaltic pump with cold (4°C) solutions of phosphate-buffered saline, pH 7.4, followed by 4% paraformaldehyde and 0.1% glutaraldehyde. All solutions were delivered at a rate of 100 ml/3 min. The brains were removed and blocked into 5- to 7-mm sections containing the injection sites and stored in the paraformaldehyde-glutaraldehyde solution for 7 days. The brains were then cut into 30-µm sections on a Vibratome and every fifth section was saved and stained with cresyl violet. Coronal sections were taken from a region located approximately 2.2 to 2.4 mm posterior to bregma. Quantitative assessments of hippocampal morphology were made using a semiautomated image analysis system (Jandel SigmaScan). Representative sections (two per brain) of dorsal HPC, matched according to stereotaxic plane and location, were projected at a magnification of 75X onto a digitizer pad interfaced with a Zenith Data Systems computer. Seven hippocampal landmarks were measured to the nearest 1 µm on the projected images. These areas (see Fig. 6), defined according to previous studies of hippocampal morphology, included (1) the distance from

FIG. 3. Time course of changes in ODC activity following injection of kainic acid into the left HPC. Rats were sacrificed 6, 12, 72, or 216 h following the injection. The data are presented as percentages (mean ± SEM) of the ODC activity in the control group which was injected with CSF. *P < 0.01 vs controls, Scheffé test.
stratum oriens overlying the CA1 pyramidal cell field to the crest of the dentate molecular layer of the superior blade; (2) the width of the dentate molecular layer of the superior blade and (3) the inferior blade, consisting primarily of apical and basilar dendrites of the dentate granule cells; (4) the width of the CA1 pyramidal cell layer consisting of pyramidal cells; (5) the width of the granule cell layer in the superior blade and (6) the inferior blade, consisting of densely packed aggregates of granule cells; and (7) the pyramidal cell layer of CA3 which was taken as the most lateral extent of CA3. All measurements were made perpendicular to the orientation of the cell layers.

Intrahippocampal injection of either KA or COL produced a selective profile of hippocampal damage (Fig. 6). One-way ANOVAs followed by post hoc comparisons revealed that intrahippocampal colchicine produced a significant 32% decrease in the width of the molecular layer of the superior blade of the dentate gyrus (Measure ii; \( F(4,43) = 7.88, P < 0.0001 \)) and a 48–53% decrease in the width of both the superior blade (Measure v; \( F(4,43) = 21.4, P < 0.0001 \)) and the inferior blade (Measure vi; \( F(4,43) = 39.2, P < 0.0001 \)) of the dentate gyrus. Post hoc analyses revealed that ganglioside GM1 did not attenuate any of these histological alterations. KA produced a selective 56% decrease in the width of the CA3 cell field (\( F(4,43) = 39.2, P < 0.0001 \)). Again, ganglioside GM1 did not attenuate the histological changes induced by KA. Neither of the cytotoxins altered Measures i, iii, or iv; all \( F \)'s(4,43) < 1.5, all \( P \)'s > 0.05 (Table 1).

**DISCUSSION**

The experiments presented here demonstrate that unilateral damage to specific populations of hippocampal neurons results in increased activity of ODC, the rate-limiting enzyme for the synthesis of the polyamines putrescine, spermine, and spermidine. Microinjection of the cytotoxins, COL and KA, produced significant increases in ODC activity in both the injected and noninjected HPC. The magnitude as well as the time course of these changes varied with the cytotoxin, the site of injection, and whether or not the animals had been pretreated with ganglioside GM1. Furthermore, even the in-

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**FIG. 4.** Effects of pretreatment with 30 mg/kg GM1 ganglioside (C-GM1) or saline (C-SAL) on the increase in ODC activity produced by unilateral intradentate injection of COL. Rats were sacrificed 24 h following injection. GM1 had no effect on ODC activity following injection of CSF into the HPC so a single control group (CON) consisting of rats pretreated with either GM1 or saline was created. The data are presented as percentages of control activity: (injected side = 1338 ± 163 pmol/mg protein/h; noninjected side = 337 ± 39 pmol/mg protein/h). See text for further experimental details. *\( P < 0.01 \) vs CON, Student's \( t \) test.

**FIG. 5.** Effects of pretreatment with 30 mg/kg of GM1 ganglioside (KA-GM1) or saline (KA-SAL) on the increase in ODC activity produced by unilateral injection of kainic acid into the CA3 pyramidal cell field. Rats were treated for 3 consecutive days with 30 mg/kg of GM1 ganglioside or saline prior to surgery and were then sacrificed 24 h following the intrahippocampal injection of kainic acid. Data are presented as percent of control activity. A single control group (CON) was created as described in the legend to Fig. 4 (injected side = 225 ± 29 pmol/mg protein/h; noninjected side = 174 ± 24 pmol/mg protein/h). *\( P < 0.05 \) vs CON, **\( P < 0.01 \) vs CON and KA-SAL group, Student's \( t \) test.
FIG. 6. Photomontage illustrating the histological damage induced by intrahippocampal injection of 0.2 µg of kainic acid (A) or 3.5 µg of colchicine (B) into the left hippocampus. These cresyl violet-stained sections demonstrate that kainic acid produced an extensive loss of pyramidal neurons in CA3, while colchicine produced a loss of granule cells in the dentate gyrus and an overall decrease in hippocampal volume (see Table 1 for further comparisons).

Injection of small volumes (0.5 µl) of artificial CSF, an osmotic and ionic balanced solution, into either the dentate gyrus or CA3 produced a transient increase in ODC activity. These data demonstrate that hippocampal damage results in increases in the activity of ODC at the site of injection, but also at points distal to the primary damage in the contralateral HPC. Since the two hippocampi are anatomically interconnected by an extensive system of commissural fibers (21) it is likely that insult to one HPC induces either direct or transynaptic alterations (i.e., transneuronal degeneration) in the other (11, 13). Furthermore, the contralateral HPC contributes to the synaptic reorganization that occurs following unilateral damage to this structure or to its afferent input (12, 23). Other studies in which one HPC was removed (15) or the hilus of one dentate gyrus was electrolytically damaged (5) also found a large and persistent increase in ODC activity on the side contralateral to the damage. These types of neural insult are difficult to quantify and are likely to affect a variety of neural elements in the HPC including granule cells, pyramidal cells, interneurons, and both association and commissural fibers. Therefore, cytotoxins that can selectively destroy a population of hippocampal neurons may provide greater opportunity to define the relationship between the extent and specificity of hippocampal damage and the response of ODC.

The second series of experiments demonstrated that injection of the putative neurotrophic factor ganglioside GM1 for 3 days prior to surgery produced complex changes in the response of the HPC to injury. In the COL group the GM1 regimen attenuated the ODC response on the injected side while enhancing the contralateral response. The topographic effects of GM1 on COL-induced changes in ODC activity may reflect a dual mechanism of action for the ganglioside whereby the metabolic consequences of the primary insult are reduced on the injected side due to a "protective" effect and neural reorganization is promoted in the contralat-
eral HPC due to a "neurotrophic" action. However, despite the fact that GM1 promotes recovery of behavioral function following intradentate COL it does not limit the effects of COL on gross indices of hippocampal damage such as decreases in the size of the granule cell layer and the molecular layer in both the superior and inferior blades of the dentate gyrus (18, 36).

While a dual mechanism of action for GM1 is attractive in light of the COL data, it is difficult to reconcile with the fact that GM1 enhanced the ipsilateral but not the contralateral response to KA. The mechanism responsible for the primary insult may be a relevant factor in the ultimate response of ODC. COL destroys vulnerable populations of neurons by disrupting axoplasmic transport (20, 32), while KA interacts with specific excitatory amino acid receptors (26) to induce cell death through a mechanism involving excitotoxicity. Thus the effect of GM1 on ODC activity following KA might relate to the involvement of excitotoxic mechanisms. Polyamines themselves have been shown to stimulate the calcium-stimulated release of the excitatory neurotransmitter aspartate (8). A cascade of neurotoxic effects may involve GM1-enhancing polyamine synthesis at the site of injury and this in turn may promote the release of excitatory amino acids. In this context Paschen and colleagues (27) suggest that increased polyamine synthesis contributes to the delayed neuronal loss observed following reversible forebrain ischemia (but also see (10)).

Morphometric analysis of the hippocampus revealed that each cytotoxin produced a very specific pattern of hippocampal damage with COL selectively decreasing the size of the dentate granule cell layer and the overlying molecular layer, which consists of the dendrites of granule cells, and KA decreasing the size of the CA3 pyramidal cell layer. Pretreatment with ganglioside GM1 did not alter the profile or magnitude of the hippocampal injury induced by the cytotoxins. Thus, the ganglioside treatment did not appear to exert a "protective" effect on the target populations of neurons. There was compara­ble hippocampal damage regardless of pretreatment with GM1, as determined by the measures used in this study. Therefore, it is likely that gangliosides either spare neurons which are interconnected with the dam­aged areas or promote synaptic reorganization such as sprouting.

Studies that further examine the role of polyamines in neuronal cell death and neural reorganization and their interaction with trophic factors should help to elucidate the biological substrates of neuroplasticity. Furthermore, the use of the HPC as a model system and selective cytotoxic agents would seem a useful approach to characterize the nature and specificity of the ODC response following neural damage.

ACKNOWLEDGMENT

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REFERENCES


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**TABLE 1**

Morphometric Analysis of the Effects of Ganglioside GM1 on the Hippocampal Damage Induced by Kainic Acid or Colchicine

<table>
<thead>
<tr>
<th></th>
<th>Controls*</th>
<th>Col-Sal</th>
<th>Col-GM1</th>
<th>KA-Sal</th>
<th>KA-GM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CA1-2 (stratum oriens + pyramidal layer + stratum radiatum + stratum lacunosum molecular)</td>
<td>670.0 ± 15.5</td>
<td>656.7 ± 12.6</td>
<td>645.1 ± 16.3</td>
<td>655.2 ± 14.6</td>
<td>642.4 ± 13.3</td>
</tr>
<tr>
<td>2. Molecular layer of dentate gyrus (superior blade)</td>
<td>245.4 ± 10.4</td>
<td>169.2 ± 12.8*</td>
<td>162.7 ± 12.9*</td>
<td>253.3 ± 6.7</td>
<td>239.8 ± 12.9</td>
</tr>
<tr>
<td>3. Molecular layer of dentate gyrus (inferior blade)</td>
<td>215.4 ± 4.7</td>
<td>198.5 ± 16.1</td>
<td>203.7 ± 7.5</td>
<td>218.0 ± 11.6</td>
<td>229.8 ± 10.6</td>
</tr>
<tr>
<td>4. Pyramidal cell layer of CA1–CA2</td>
<td>70.3 ± 2.7</td>
<td>34.8 ± 4.0*</td>
<td>35.2 ± 4.0*</td>
<td>66.8 ± 6.0</td>
<td>72.2 ± 6.9</td>
</tr>
<tr>
<td>5. Dentate granule cell layer (superior blade)</td>
<td>68.8 ± 3.0</td>
<td>32.5 ± 4.9*</td>
<td>36.0 ± 3.2*</td>
<td>66.8 ± 6.6</td>
<td>56.7 ± 4.4</td>
</tr>
<tr>
<td>6. Dentate granule cell layer (inferior blade)</td>
<td>128.0 ± 14.1</td>
<td>133.0 ± 6.1</td>
<td>133.2 ± 14.9</td>
<td>67.2 ± 9.2*</td>
<td>50.7 ± 4.8*</td>
</tr>
</tbody>
</table>

* Analyses of variance revealed that all of the measures of hippocampal morphology on the noninjected side were comparable for all the treatment groups. Therefore, the data were combined to form a single group designated as controls (N = 24). All of the other groups had an N of 6.

* P < 0.05 vs controls, Scheffe's test.


