

EARLY CHANGES IN ORNITHINE DECARBOXYLASE ACTIVITY IN A PARTIALLY DENERVATED HIPPOCAMPUS OF RATS UNTREATED AND TREATED WITH GM₁ GANGLIOSIDE

M. SKUP and M. GRADKOWSKA

Department of Neurophysiology, Nencki Institute of Experimental Biology,
3 Pasteur St., 02-093 Warsaw, Poland

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Abstract. Bilateral transection of the lateral fimbria, which disrupts partially the septo-hippocampal projections and results in partial hippocampal denervation, produced a significant increase in the ornithine decarboxylase (ODC) activity in the hippocampus. An increase occurred already 0.5 h after the operation and the activity remained intensified for at least 22 h after injury. The enzyme response was enhanced by a single dose of GM₁ monosialoganglioside (30 mg/kg) administered directly after the operation. This enhancement, detected 2 h after the injury, persisted for at least 22 h after the operation. Lack of influence of GM₁ ganglioside on ODC activity in the hippocampus of unlesioned animals allows us to ascribe the observed effect to the processes induced by the lesion. This study confirms the involvement of ODC in GM₁ ganglioside neurotrophic effects produced in an injured brain.

INTRODUCTION

Ornithine decarboxylase (ODC) is a highly inducible, first and rate-regulating enzyme of polyamine synthesis, which play an important role in neuronal survival and regeneration (6, 8, 16, 27, 28). The activity of this enzyme can be modulated by a variety of extrinsic factors such as

aminoacids and hormones (15, 19, 26). It has also been shown that the nerve growth factor (NGF) rapidly induces the enzymatic ODC activity in some neuronal cell types *in vitro* (7, 11, 20, 24) and that its injection at the proximity of the peripheral nerve endings (13) as well as its administration to the brain (17) of an adult rat results in a marked increase in ODC activity in the neuronal tissue. Several data indicate that also gangliosides, which seem to act as trophic factors in the nervous system, increase the ODC activity when added to cell and tissue cultures (23, 24). Recently it has been shown by Zini et al. (32) that polyamines may play a permissive role in the central nervous system (CNS) with regard to the trophic action of GM₁ ganglioside when administered to partially hemitranssected rats. The above authors proved that the GM₁ trophic effects in that model system can be attenuated by the administration of difluoromethylornithine, a specific inhibitor of ODC; the GM₁ effect can then be reinstated by direct administration of putrescine. To investigate further the question of the mechanism of the GM₁ ganglioside action in the injured nervous system, the model of partially denervated hippocampus obtained by a lateral fimbria transection was applied and investigations were made of the effect of GM₁ administration on ODC activity in the hippocampus of the intact rats and of the rats operated on. Using the model of hippocampal denervation (4, 10, 21, 22) we had proved previously a significant attenuation of post-injury changes in the hippocampus after a short-term GM₁ ganglioside treatment.

MATERIAL AND METHODS

Reagents. DL-[1-¹⁴C] ornithine monohydrochloride (58 mCi/mmol) was purchased from Amersham International (Amersham, Bucks, UK); sodium pentobarbital (Nembutal) was obtained from the Abbot Laboratories, North Chicago, Ill., USA; phenylethylamine was purchased from New England Nuclear and dithiotreitol (DTT) from British Drug Houses Ltd., Laboratory Chemical Division, Poole, UK; pyridoxal 5'-phosphate and ornithine were obtained from Sigma Chemical (St. Louis, Mo, USA). All the other chemicals were analytical grade products.

GM₁ ganglioside: product of purity over 99% obtained from Fidia S.p.A. (Abano Terme, Italy) was used.

Animals, surgery and treatment. Adult, male Wistar rats, weighing 200-240 g were used. The animals had free access to food (standard pellets) and tap water. Before the operation the animals were anesthetized with sodium pentobarbital (Nembutal; 50 mg/kg, i.m.) and then operated on by a bilateral knife-cut of the lateral fimbria made stereotaxically and resulting in a partial transection of the septo-hippocampal

pathways (Fig. 1). Two groups of lesioned rats were studied: untreated and treated with GM₁ ganglioside. A single dose (30 mg/kg, i.m.) of GM₁ ganglioside was injected immediately after operation, which lasted ca 20 min.

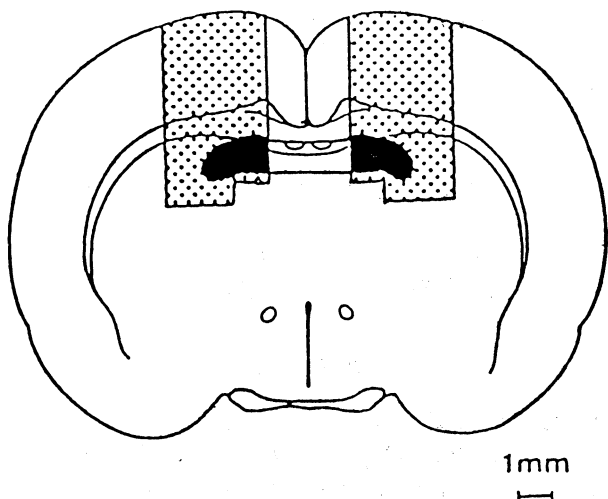


Fig. 1. Bilateral, partial transection of the lateral fimbria. The area of knife manipulation dotted; transected fimbria marked in black.

Three groups of control rats, not operated on, were used: untreated, anesthetized only, and anesthetized, successively injected with GM₁. The treatment schedule and the doses of Nembutal and of GM₁ ganglioside were the same as those applied in the groups operated on. The animals were killed by decapitation at 0.5 h, 2 h, 4 h or 22 h after respective treatment.

Tissue preparation. The brains were quickly removed from the skulls and cooled, and the hippocampi were isolated. The hippocampi from each brain were pooled and homogenized in 8 volumes of ice-cold 25 mM Tris-HCl bufer (pH 7.4) which contained pyridoxal 5'-phosphate, DTT and EDTA. The homogenates were centrifuged at $48,000 \times g$, for 20 min, 4°C. The resulting supernatants were used for estimating the ODC activity and protein content.

Ornithine decarboxylase assay. Ornithine decarboxylase (E.C. 4.1.1.17) activity was estimated by the radiometric method of Russel and Snyder (25), modified according to Slotin and Bartholome (26), with the use of DL-[1-¹⁴C] ornithine. An incubation mixture (45 μ l) which contained 25 mM Tris-HCl (pH 7.4), 5 mM DTT, 5 mM ornithine plus 2 mM ¹⁴C ornithine (f.c. of substrate 200 μ M) was added to 200 μ l of supernatant,

and after the addition of pyridoxal 5'-phosphate to f.c. 0.1 mM, the samples were incubated for 1 h in sealed tubes in a shaking water bath at 37°C. The labeled $^{14}\text{CO}_2$ which was released during the reaction was trapped on a paper wick (dripped with 140 μl of phenylethylamine) suspended over the reaction mixture in a plastic well attached to a rubber seal. The blanks contained, instead of a supernatant, the homogenization buffer. The reaction was stopped and CO_2 was released with 0.5 ml of 10% trichloroacetic acid during 30 min of postincubation in 37°C. The filters were then removed and a $^{14}\text{CO}_2$ trapped was counted in a scintillation counter. The ODC activity was expressed as pmoles of CO_2 released per hour per mg of protein. All the estimations were made in triplicate.

The protein content was assayed according to the modified Lowry method as described by Markwell et al. (18).

RESULTS

In the intact control rats the level of ODC activity in the hippocampus amounted to 12.91 ± 0.809 pmoles/mg prot./h. The administration of Nembutal resulted in a small, nonsignificant decline (15-20%) in the enzyme activity within the initial 2 h after treatment (Fig. 2) while 4 h

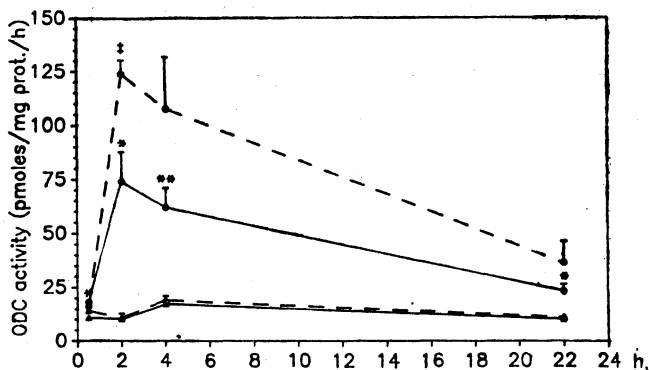


Fig. 2. The effect of GM₁ ganglioside administration (single dose, 30 mg/kg) upon the changes of ODC activity in the hippocampus of rats within first day following bilateral transection of the lateral fimbria. Control, Nembutal injected rats (triangles, solid line); control, Nembutal injected and GM₁-treated rats (triangles, dashed line); lesioned, untreated rats (circles, solid line); lesioned, GM₁-treated rats (circles, dashed line). Results are expressed in pmoles of CO_2 formd/mg protein/h and are means \pm SEM from 4-9 rats in each group. Values obtained in intact, control animals ($n = 36$) are 12.91 ± 0.809 pmol/mg protein/h. Differences between the values in control and lesioned, untreated rats (*) and between the values in lesioned, untreated and lesioned, GM₁ treated rats (†) significant at: ** $p < 0.001$.

* $p < 0.05$, † $p < 0.05$ (Student's t -test).

after the Nembutal injection there was a 30% increase ($p < 0.05$) of ODC activity above the control values. At 22 h a small decline below the control values was detected. In the control rats, anesthetized and successively injected with GM₁ ganglioside, the changes of ODC activity paralleled those evoked by the administration of Nembutal only.

As shown in Fig. 2, ODC activity increased significantly as early as 0.5 h after the lesion placement. This initial increase was further intensified in the later postoperative period, reaching the maximum 2 h after operation (720% of control values in unlesioned, Nembutal injected animals). The activity was still higher after 22 h as compared with the controls.

GM₁ ganglioside administration elevated significantly the level of ODC activity induced by the lesion. The effect of GM₁ ganglioside treatment, not detected at 0.5 h, peaked at 2 h after the operation (1120% of the control values in unlesioned, GM₁ injected animals, and 170% as compared with the values from lesioned rats) and persisted for at least 22 h after injury.

The analysis of variance performed for the data which concerned the postoperative ODC alterations in the hippocampus revealed a high statistical significance of the changes in all three parameters: time ($p < 0.001$), operation ($p < 0.001$, as compared with the unlesioned groups) and GM₁ treatment ($p < 0.02$, as compared with the lesioned, untreated groups). Significant interactions have been found between the time and the operation factor ($p < 0.001$) as well as between the operation and the GM₁ factor ($p < 0.05$) but not between the GM₁ ganglioside and time factor.

DISCUSSION

The present results demonstrate that the activity of ODC increased dramatically in the hippocampus within the first few hours after a bilateral lesion of the lateral fimbria. This increase persisted at least through the first postoperative day. In the light of the previous findings (1, 2, 5, 6, 28-32), the postinjury stimulation of ODC activity seems to be a widespread phenomenon; our results confirm this assumption.

Our data indicate also that none of the treatments applied to unlesioned control animals induced markedly ODC activity within the investigated period. A slight decrease of the level of the ODC activity observed within two hours after a Nembutal injection, followed by a transient increase in ODC activity at 4 h, should be considered rather as a consequence of some anesthesia-evoked disturbances in the neuronal activity, which may provoke the enzyme response.

It should be mentioned that sham operation that omitted the septo-hippocampal projections but damaged extensively the cortical tissue caused an increase of ODC activity (data not shown) higher than that found in unlesioned, Nembutal injected controls. However, this increase was much lower in comparison with that occurring after fimbrial transection. Nevertheless, it is obvious that this kind of sham operations make animals suffer some tissue damage and a hemorrhage, which would lead to the induction of a heat-shock response (6, 9).

The detected postlesion changes in the ODC activity are very rapid. For comparison, onset, reported by Desiderio et al. (5), of the ODC changes in the substantia nigra and in the striatum after hemitransection of the nigro-striatal pathways appears later. However, the pattern of changes in this model resembles that found by us, namely, our recent data obtained in the septum (not shown) also indicate that the maximal increase of the ODC activity in the projecting structure precedes the increase detected in the target area.

ODC changes found by other authors in the hippocampus of rats after hilar lesion (1) reveal a different profile, reaching the maximum 12 h after the operation. On the other hand, the time courses of the ODC increase in the nuclei and in the ganglia of the axotomized peripheral nerves (28, 30, 31) resemble those described for the neurons of the CNS, although the distances between the lesion placement and the denervated areas differ significantly. Therefore, these similarities and differences in the velocity of the development of ODC activation cannot be explained only by the various distances of the structures investigated from the place of injury.

The fact that irrespectively of the differences in the onset and degree of postinjury ODC activation this process is generally very rapid in all the cases discussed strengthens the suggestion of ODC involvement in the regulation of early postinjury processes in the brain. These include genomic expression (12) and protein phosphorylation (3) followed by cytoskeleton structural changes, all these processes being early symptoms of cellular mobilization and growth.

It should be underlined that ODC activity changes cannot be considered as a neuronal event only. For example, Wells reported that post-injury spontaneous ODC activation in the periphery occurred both in the neurons and in the glial cells (30). It has been found also that NGF administration to the brain results in ODC increase both in the neuronal and in the glial fraction, a greater increase being observed in glia (17). It remains to be established to what extent these two components are involved in the observed changes of the ODC activity.

In our study we have found that GM₁ administered even in a single

dose evoked an elevation of injury-induced ODC activity. In contrast to the results reported by Zini et al. (32) for the intact rats, we have not detected any influence of GM₁ ganglioside injection on the ODC activity either in unlesioned, Nembutal injected rats or in the unlesioned rats injected with GM₁ ganglioside only (data not shown). This result suggests that the GM₁ ganglioside effect on the ODC is mediated by the mechanism that is induced by the injury.

The augmentation by the GM₁ ganglioside of ODC induction in the rats operated on partially confirms other data (29, 32) on GM₁ enhancing effect on ODC activation in the brain nervous tissue after injury. However, while in the hemitranssection model (32) the stimulating effect of GM₁ concerned the projecting structure (nigra) only, in our model we observed the effect induced by GM₁ within the first postoperative day at the target level. Although the reason of this discrepancy is unclear, our result points to the importance of the processes occurring at the target level in terms of ODC/GM₁ interactions after transection of the septo-hippocampal pathways. In our model it has been previously shown that the GM₁-enhanced early recovery of the hippocampal biochemical parameters of cholinergic and serotonergic systems is due rather to protection against secondary degeneration than to the acceleration of neuronal growth, i.e. reinnervation processes. In this case the ODC involvement, reported by Jensen et al. (14), in the homeostasis by regulation of injury-induced Ca⁺² overloads would be of special importance: GM₁ enhancement of ODC activation would facilitate maintenance of ionic balance in injured system.

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