ANALYSIS OF THE TIME COURSE OF GM₁ GANGLIOSIDE EFFECT ON CHANGES IN CHOLINE ACETYLTRANSFERASE ACTIVITY IN PARTIALLY DENERVATED RAT HIPPOCAMPUS

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Abstract. The effect of chronic GM₁ ganglioside administration (30 mg/kg, daily) for 6, 21, 42 and 90 days on the activity of choline acetyltransferase was investigated in the hippocampus of rats with partial electrolytic lesions of the dorsal hippocampal afferents and in unoperated rats. No influence of GM₁ administration on ChAT activity was noted in unoperated animals. The lesions caused denervation in the hippocampus, which occurred with varying intensity along its dorso-ventral axis, as shown by the gradual pattern of decrease in ChAT activity. GM₁ counteracted the decline in enzyme activity, however the intensity of this influence diminished with the time after surgery. A positive correlation between the GM₁ effectiveness and the degree of denervation at early postsurgical stages (6, 21 days) was found, which may be ascribed to the appearance of neuronotrophic factors at this period, proportional to the severity of damage. We suggest that the decline of the GM₁ effectiveness is due to a decrease in trophic activity, and/or the development of spontaneous recovery.

INTRODUCTION

The contribution of gangliosides, in particular GM₁ monosialoganglioside, to processes of recovery in an injured central nervous system was extensively investigated in the past few years. Lesion studies com-
bined with GM1 administration, brought evidence that GM1 facilitates the repair of various forms of morphological, behavioral and biochemical postlesion impairments (1, 8, 14, 24, 27-29). In particular, numerous papers report a GM1 effect on the recovery of biochemical markers of different brain neurotransmitter systems (1, 3, 13, 15, 16, 22, 23, 29, 30).

Recently, a convenient system permitting simultaneous monitoring of the GM1 effect upon postlesion alterations in various denervation conditions has been applied in our laboratory (12, 21). Namely, by performing partial lesions of dorsal hippocampal afferents, different degrees of degeneration of the cholinergic fibers along the dorso-ventral axis of the hippocampus are obtained. Using this strategy, we have been able to show the early effect of GM1, expressed in attenuation of diminished levels of ChAT, AChE and HAChU in the hippocampus, proportional to the degree of cholinergic fibre degeneration (12, 21). The aim of this paper is to present a detailed analysis of this phenomenon after various postlesion periods (from 6 to 90 days) for ChAT activity, the most reliable presynaptic cholinergic marker. In addition, an analysis of the GM1 effect upon ChAT activity in the hippocampus in the unlesioned brain after various periods of treatment (from 6 to 90 days) has been also performed.

METHODS

Materials. GM1 ganglioside of purity over 99% was kindly supplied by the Fidia Research Laboratories, Abano Terme, Italy. 1-14C acetyl-coenzyme A (S.A. 55mCi/nmol) was purchased from Amersham International Ltd., UK. All the other reagents were of analytical grade.

Animals, surgery and treatment. Male Wistar rats, 3 months old, weighing 200-240 g were used. Bilateral electrolytic lesions were performed under sodium pentobarbital anesthesia (50 mg/kg, i.m.). An anodal current (2.5 mA) was passed for 30 s through a tungsten electrode. All lesions were made within the level from 7890 μm to 6360 μm anterior to the interaural-line, as shown on consecutive schemes in Fig. 1 (left), and destroyed, to various extents, the area through which run the dorsal hippocampal pathways. The animals were housed in groups of four with free access to water and food. Four groups of animals were run simultaneously: unoperated and operated GM1-injected, unoperated and operated vehicle-injected. GM1 was dissolved prior to injection in 0.01 mol/l phosphate buffer, pH 7.4, being used as the vehicle. GM1 was given chronically once daily (30 mg/kg i.m. — thigh muscle)
Fig. 1. Examples of partial dorsal hippocampal pathway lesions (marked hatched) are shown on drawings adapted from the Koenig and Klippel atlas, representing the frontal sections through the brain from rostral (A 7890 μm) to ventral (A 6360 μm) levels. Lesions encroached more posteriorly (A) or more anteriorly (B) on the supracallosal and adjacent areas. C, cingulum; CA, commissura anterior; CFV, commissura fornicis ventralis; F, columna fornicis; FO, fornix; FS, fornix superior; sl, nucleus septi lateralis; sm, nucleus septi medialis; SM, stria medullaris; TCC, truncus corporis callosi.
in 0.2 ml of the buffer, beginning on the first day after the surgery, for a period of 6, 21, 42 or 90 days. The animals were killed by decapitation the day after the last GM₁ dose. All four groups were in good health conditions.

Dissection of tissue and histological verification. After decapitation, the brains were speedily removed from the skulls, left and right hippocampi dissected out on ice and divided perpendicularly to the longitudinal hippocampal axis into 3 consecutive, equal parts (I, II, III). Then, the corresponding pieces of each two hippocampi were pooled and homogenized for biochemical assay. In each operated rat the anterior part of the brain was taken for histological verification of the lesion. Coronal sections (25 μm) were made in a cryostat and stained with cresyl violet.

Biochemical estimations. All estimations were made in hippocampal homogenates in triplicate. The tissue was homogenized in 20 vol of 0.32 mol/l sucrose solution, by means of a motor-driven Potter Elvehjem glass homogenizer with a teflon pestle. Homogenates were frozen and kept at −20°C until analysis.

ChAT. The ChAT activity was estimated according to the radiometric method of Fonnum (9), with 1-14C acetylcoenzyme A, as the acetyl group donor.

Protein content. Protein content was estimated according to the modification of Lowry's method described by Markwell et al. (17).

Data analysis. Statistical analysis was performed by means of Student's t-test. For calculation of the mean correlation coefficients (r) and the confidence intervals for the absolute values of the correlation coefficients, a computer simulation program (Oderfeld and Pogorzelski, in preparation) which takes into account the dispersion of the individual data (i.e. SEM values) has been used. The principle of the method has been described in detail by Kiedrowski et al. (15). This program generates 1,000 realizations of linear regression, taking random values from the dispersion range of the respective data.

RESULTS

Lack of effect of GM₁ treatment upon ChAT activity in the hippocampus of unoperated rats. In unoperated, buffer-injected rats, the dorsoventral, ascending gradient of ChAT activity has been demonstrated, confirming previous data obtained for untreated rats (7, 26). Ganglioside treatment remained without influence on ChAT activity, irrespective of its duration and the hippocampal part analyzed (Table I).

Effect of GM₁ treatment upon changes in ChAT activity in the hippocampus of operated rats. Essentially two groups of lesions could be distin-
Lack of effect of GM₁ treatment on ChAT activity in 3 parts of the hippocampus of unoperated, control rats, over various periods of treatment. The data are mean ChAT activity expressed as μmoles of ACh × 100 mg protein⁻¹ × h⁻¹ ± SEM. I–III, hippocampal parts, from dorsal to ventral end. Buff and GM₁, buffer- and GM₁-treated groups of rats. In buffer-treated groups n = 7–12, in GM₁-treated groups n = 4–8. In all cases the differences between the values in the two groups were not statistically significant, irrespective of the hippocampal part and the period of treatment.

<table>
<thead>
<tr>
<th>Days of treatment</th>
<th>I Buff</th>
<th>GM₁</th>
<th>II Buff</th>
<th>GM₁</th>
<th>III Buff</th>
<th>GM₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>5.47±0.48</td>
<td>5.22±0.17</td>
<td>6.61±0.30</td>
<td>5.83±0.11</td>
<td>7.42±0.26</td>
<td>7.32±0.42</td>
</tr>
<tr>
<td>21</td>
<td>4.79±0.36</td>
<td>4.85±0.39</td>
<td>5.54±0.35</td>
<td>5.57±0.32</td>
<td>6.57±0.27</td>
<td>6.69±0.28</td>
</tr>
<tr>
<td>42</td>
<td>4.59±0.37</td>
<td>4.95±0.26</td>
<td>5.58±0.38</td>
<td>5.22±0.45</td>
<td>6.28±0.34</td>
<td>7.41±0.54</td>
</tr>
<tr>
<td>90</td>
<td>4.86±0.16</td>
<td>5.10±0.23</td>
<td>5.61±0.34</td>
<td>6.45±0.13</td>
<td>6.72±0.29</td>
<td>6.85±0.19</td>
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guished, encroaching more posteriorly or more anteriorly on the supraccallosal and adjacent areas, as shown in Fig. 1A and B, respectively.

Confirming our previous data (12), the posteriorly located lesions resulted in a much stronger decrease in ChAT activity in the hippocampus (Fig. 2, panel A) than those located more anteriorly (Fig. 3, panel A). The lesion evoked decrease was especially expressed in the dorsal part of the hippocampus (part I), diminishing progressively towards the ventral end (part III). Owing to the fact that subtle differences in lesion placement within each type of the lesion affected the magnitude of the decrease of ChAT activity, it was difficult to investigate in detail the time course of postlesion alterations. However, the obtained data indicate that after A type lesion (Fig. 2, panel A) the level of ChAT activity, still remaining much below control level in the most affected dorsal part (I) even at longest postoperative time, was higher in the middle part (II) and especially in the most ventral part (III) at 42 and 90 days than in the corresponding parts after 6 days (the differences between values in part II at 6 and 90 days, and for values in part III between 6 and 42–90 days were statistically significant). These results can be regarded as a sign of spontaneously occurring recovery processes. No such phenomenon was observed after B type lesion (Fig. 3, panel A).

Confirming our previous findings obtained after the early postoperative period (4, 21), a GM₁ counteracting effect upon the decreased ChAT activity was observed at 6 and 21 days after A type lesion (Fig. 2, panel B). A distinct relationship between this effect and the degree of degeneration of the injured hippocampal afferents expressed as a per-
Fig. 2. Relationship between GM₁ influence on postlesion (type A lesion compare Fig. 1) changes in ChAT activity over various periods of treatment and the degree of decrease in this marker in three consecutive (I-III) dorso-ventral hippocampal parts. Panel A, percentage change in levels of ChAT activity in operated, buffer-treated group, in comparison with values for unoperated, buffer-treated group; panel B, percentage change in levels of ChAT activity in operated, GM₁-treated rats, in comparison with values for operated, buffer-treated group. Data are means from 3-6 rats in each group.

* 0.05 < P < 0.1, ** P < 0.001, in respect to values in corresponding hippocampal part of the reference group. Student's t-test.
Fig. 3. Relationship between GM₁ influence on postlesion (type B lesion, compare Fig. 1) changes in ChAT activity over various periods of treatment and the degree of decrease in this marker in three consecutive (I-III) dorso-ventral hippocampal parts. Data are means from 3-4 rats in each group; except for 90 days where the values (A and B) represent data for single animals. All explanations as in Fig. 2.
Fig. 4. Regression lines for the dependence of GM₁ influence upon the degree of postlesion ChAT activity decrease in the hippocampus in various periods of treatment after partial lesion (type A, compare Fig. 1) of the dorsal hippocampal afferents. The inset presents the slope (s) of the regression lines in function of the time after lesion. Calculations were made for the data shown in Fig. 2. Regression equations: at 6 days: \( y = 1.07 x -18 \); at 21 days: \( y = 1.02 x -11 \); at 42 days: \( y = 0.78 x -22 \); at 90 days: \( y = 0.07 x -2 \).

**Table II**

Correlation analysis of GM₁ influence on postlesion changes in ChAT activity over various periods of treatment with the postlesion degree of decrease in this marker. For analysis combined data presented in Fig. 2 (after type A lesion) and Fig. 3 (after type B lesion) were used, the calculations were performed according to the computer simulation program (Oderfeld and Pogorzelski, see Methods)

<table>
<thead>
<tr>
<th>Days of treatment</th>
<th>Mean correlation coefficients ((r))</th>
<th>Confidence intervals at 0.9</th>
</tr>
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<tbody>
<tr>
<td>6</td>
<td>0.825</td>
<td>( 1 &gt; r &gt; 0.617 )</td>
</tr>
<tr>
<td>21</td>
<td>0.641</td>
<td>( 1 &gt; r &gt; 0.334 )</td>
</tr>
<tr>
<td>42</td>
<td>0.463</td>
<td>( 1 &gt; r &gt; 0.109 )</td>
</tr>
<tr>
<td>90</td>
<td>0.148</td>
<td>( 1 &gt; r &gt; -0.936 )</td>
</tr>
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percentage decrease of ChAT activity was noticed: the effect was seen only in the most affected hippocampal parts: I and II. The effect of GM₁ diminished with time after lesion (42 days) and disappeared completely after 90 days (Fig. 2, panel B). After B type lesion, causing only a small decrease of ChAT activity, no effect of GM₁ treatment was observed, independently of the postlesion time (Fig. 3, panel B).

The decay of GM₁ effectiveness with postoperative time after A type lesion is illustrated additionally in Fig. 4, in which the respective regression lines at various postoperative periods and their slopes as a function of postlesion time (see inset) are shown. Table II presents an analysis of the correlation between GM₁ effect and the degree of fiber degeneration for combined data presented in Fig. 2 and Fig. 3. A strong correlation was observed only on the 6th day after the lesion, moderate on the 21st day and very weak on the 42nd day.

DISCUSSION

The main finding of the present study is that the GM₁ evoked post-lesion attenuation of reduced levels of ChAT activity, dependent on the degree of hippocampal denervation, diminishes progressively with time after surgery (Fig. 4). The observed GM₁ influence is specifically related to the postlesion events, since even the long-lasting GM₁ administration (up to three months) in unoperated rats remained without any effect on ChAT activity (Table I).

The lack of GM₁ effect in unlesioned animals confirms previous data obtained in our or other laboratories concerning the short-term treatment (4, 12, 21), but differs from those reported by Cuello and his co-workers (6). The reason for this discrepancy remains obscure; one possibility is that the latter data were obtained on young animals. Our recent data indicate that also ChAT kinetic parameters do not alter after short term GM₁ administration to unoperated rats (20).

The decay of GM₁ effectiveness on ChAT activity changes in the hippocampus at longer postlesion periods, fits the results recently reported by Kiedrowski et al. (15), indicating transient GM₁ effects on hippocampal serotonergic system after denervation. As it has been suggested and discussed in this paper, the early GM₁ effect on biochemical indices after surgery may probably be due to the prevention of secondary degeneration. Supporting for this view is analysis of GM₁ postlesion influence on ChAT kinetic parameters which revealed attenuation of reduced \(V_{\text{max}}\) values and lack of GM₁ effect on \(K_m\) (20). A transient effect of GM₁ on the survival of neurons in substantia nigra after hemitransection of the nigro-striatal pathways has also been re-
ported (24). On the other hand, in several other experimental conditions, the ganglioside effect persisted up to 7-11 weeks after operation (1, 28, 30).

Although the mechanism by which gangliosides act on postlesion phenomena in the brain is still only hypothesized, what is noteworthy is the possible involvement of different trophic growth and survival factors known to be released in the denervated brain areas (2, 5, 18, 19). As it has been hypothesized in our previous study (12, 21), the dependence of the GM₁ effect upon the degree of fiber degeneration seen after shorter postlesion periods: 6 and 21 days, may be due to the fact that a larger quantity of such factors is released in more extensively denervated parts of the hippocampus.

As the release of neuronotrophic factors after brain injury is confined only to the short, 2-3 postsurgical weeks postlesion period (11, 19, 25), the mediation of factor activity by GM₁ is conceivable only in the early postoperative phase. The phenomenon of gradual decrease of trophic activity in the denervated area may thus explain the decline of GM₁ activity in time, found in this study. It is also not excluded that the spontaneously occurring compensatory processes (see Fig. 2, panel A) could weaken the phenomena guided by GM₁. The quantity of released neuronotrophic factors and/or the intensity of spontaneously occurring compensatory processes may thus determine the duration of ganglioside treatment effects in various experimental paradigms.

The authors are grateful to Professor J. Oderfeld and Dr A. Pogorzelski for preparing the computer program and help in elaboration of our data, and to Fidia Research Laboratories, Abano Terme, Italy, for kindly providing GM₁ ganglioside. This investigation was supported by Project CPBP 04.01 of the Polish Academy of Sciences.

ABBREVIATIONS

ACh acetylcholine
AChE acetylcholinesterase
ChAT choline acetyltransferase
GM₁ monosialoganglioside
HACHU high-affinity choline uptake

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