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Journal
Neuroscience Letters, 84(2)

ISSN
0304-3940

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Publication Date
1988-01-22

DOI
10.1016/0304-3940(88)90413-2

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GM$_1$ ganglioside enhances synaptosomal resistance to chemically induced damage

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(Received 30 July 1987; Revised version received 25 September 1987; Accepted 2 October 1987)

Key words: Ganglioside; Monosialoganglioside; Synaptosome; Calcium level; Regeneration; Neurotoxicity

A neurotoxic agent, chlordecone, damages the limiting membrane of isolated synaptosomes prepared from rat brain. This is shown by increased levels of free, ionic calcium and increased permeability of synaptosomes, using the fluorimetric probe, fura-2. Pretreatment of synaptosomes with the monosialoganglioside GM$_1$, attenuates the effect of chlordecone. A parallel preincubation of synaptosomes with diasialoganglioside GD$_{1a}$ effects a similar mitigation of chlordecone-induced elevation of free calcium but does not prevent general membrane leakiness as assayed by escape of dye from synaptosomes. These data may underlie the known effect of gangliosides in enhancing neuronal regeneration after lesion induction.

Gangliosides have been shown to accelerate regenerative phenomena in the central nervous system. Lesions, induced after ganglioside GM$_1$ treatment, which recover more rapidly, as judged by both morphological and behavioral criteria, include chemical or physical insults to the hippocampus [26], striatum [1] and cholinergic forebrain nuclei [4] and serotonergic neurons [12]. When injected systemically, a small proportion of gangliosides can enter the central nervous system in an intact form [16]. Results from whole animals on ganglioside-effected increased outgrowth of neural elements are paralleled by data from isolated neuronal cultures. In this case, gangliosides have been found to enhance neuronal sprouting [18, 19, 23, 25]. We have examined the effect of GM$_1$ ganglioside upon the response of isolated nerve endings to a neurotoxic agent known to damage the plasma membrane, causing both synaptosomal lysis and increased levels of ionic calcium [Ca$^{2+}$], within the surviving synaptosomes [5]. Under such circumstances, pretreatment with GM$_1$ exerts a protective effect upon synaptosomal integrity. The agent used, chlordecone (decachlorooctahydro-1,3,4-methano-2H-cyclobutapentalene-2-one) is a cuboidal, inert, hydrophobic chemical formerly used as an insecticide [15].
Adult male Sprague-Dawley rats, 2-3 months old weighing 200-330 g were decapitated, the brain quickly excised on ice and the whole brain except the cerebellum and pons-medulla dissected out. Synaptosomes were made by the modification of Dodd [5] of the method of Gray and Whittaker [7].

Free intrasynaptosomal \( \text{Ca}^{2+} \), \([\text{Ca}^{2+}]_i\), was measured using the acetoxymethyl ester of fura-2 (fura-2/AM) [14]. The basis of this method is the diffusion of this ester into cells or synaptosomes, followed by hydrolysis and intracellular trapping of the anionic dye. The calcium salt of this material can then emit a characteristic fluorescent signal [8]. Samples contained 140–160 µg protein in a buffer consisting of (mM): NaCl 125, KCl 5, MgCl\(_2\) 1.2, CaCl\(_2\) 0.1, NaHCO\(_3\) 5, glucose 6, HEPES 25, at a pH of 7.4. The suspension was allowed to equilibrate at 37°C for 10 min before addition of chemicals. A correction was made for any fura-2 leaking out of the particulate fraction by centrifuging synaptosomes down and determining fluorescence in the supernatant. In order to calculate \([\text{Ca}^{2+}]_i\) before addition of chlordecone, a separate correction was made for each batch of synaptosomes. This was around 6% of the fluorescence at 340 nM. For calibration of the synaptosomes fura-2-Ca\(^{2+}\) signal (R), \(R_{\text{min}}\) (the ratio of fluorescence at 340 nm/380 nm in the absence of Ca\(^{2+}\)) and \(R_{\text{max}}\) (the ratio when all fura-2 of the sample was saturated with Ca\(^{2+}\)) were determined for each batch of fura-2-loaded synaptosomes [14]. \([\text{Ca}^{2+}]_i\) was calculated using the formula of Grynkiewicz et al. [8].

Treatment of synaptosomes with chlordecone caused a large time- and dose-dependent increase in \([\text{Ca}^{2+}]_i\), together with an elevated leakage of hydrolysed fura-2 out of the synaptosome [14]. Preincubation of synaptosomes with 50 µM GM\(_1\) ganglio-

![Fig. 1. The effect of ganglioside GM\(_1\) on chlordecone-induced synaptosomal damage. The increase in concentration of free calcium within synaptosomes and the amount of fura-2 present in the incubation medium were determined 15 min after addition of chlordecone. In some experiments 5 × 10\(^{-5}\) M GM\(_1\) was added 5 min prior to chlordecone. The basal resting level of \([\text{Ca}^{2+}]_i\) was 319 ± 8 nM (n = 34). Increase of synaptosomal \([\text{Ca}^{2+}]_i\) in 15 min in the absence of chlordecone or gangliosides was 30 ± 5 nM (n = 22) and fura-2 leakage was 19.5 ± 0.9% (n = 34). Data are the means of determinations on 5 separate rat brains. Bars represent S.E.M.](image-url)
side at 37°C for 5 min prior to chlordecone addition reduced both of these indices of synaptosomal damage (Fig. 1). This suggested that pretreatment of synaptosomes with GM1 rendered them more resistant to damage by chlordecone. Ganglioside GM1 alone elevated basal levels of [Ca^{2+}] in synaptosomes significantly by 33.2 ± 11 nM (n = 24, P < 0.01), when present at 50 µM concentration. GM1 has recently been reported to participate in calcium channel function [6] and this enhancement of channel opening may be related to our finding of a minor increase in [Ca^{2+}], in synaptosomes exposed to GM1. GM1 also caused a very minor increase in the leakage of fura-2 out of synaptosomes, by 3.2 ± 1.7% (n = 19).

A parallel study using the disialoganglioside GD1A was also carried out. This ganglioside mitigated the effect of chlordecone upon synaptosomal [Ca^{2+}], but the increased leakage of dye caused by chlordecone, was not protected against (Table I). In fact, GD1A alone caused a major elevation of fura-2 leakage (Table I). Thus the effects on [Ca^{2+}], and on dye leakage appear to be independent.

At the physiological level, gangliosides have been shown to protect brain slices from hypoxia [11], and GM1 has been stated to "improve the membrane's ability to adjust to new working conditions created by a high frequency impulse train" [27]. Such events may also be related to a restriction of damage-induced calcium entry similar to that reported here. Taken together, the data imply a direct stabilization of the synaptosomal external membrane by GM1 ganglioside. This ganglioside species is potent in causing membrane lipid chains to assemble in a more rigid manner, as judged by electronparamagnetic resonance techniques [3] or by increased fluorescence polarization [10, 28]. A small proportion of GM1 present in the incubation medium is actually incorporated into the structure of the external membrane [24]. GM1 is very similar to GD1A in its effect on membrane ordering [9]. The distinctive effect of GM1 reported in vivo may be more related to its stability or access to the brain when administered systemically. The finding that GD1A greatly increased synaptosomal leakage of fura-2 (unlike GM1) suggests that this ganglioside may also have deleterious effects on the integrity of the normal synaptic membrane.

In the presence of xenobiotic agents such as ethanol, which increases the fluidity of cell membranes, GM1 may paradoxically enhance the reduction of membrane micropolarization techniques [3] or by increased fluorescence polarization [10, 28]. A small proportion of GM1 present in the incubation medium is actually incorporated into the structure of the external membrane [24]. GM1 is very similar to GD1A in its effect on membrane ordering [9]. The distinctive effect of GM1 reported in vivo may be more related to its stability or access to the brain when administered systemically. The finding that GD1A greatly increased synaptosomal leakage of fura-2 (unlike GM1) suggests that this ganglioside may also have deleterious effects on the integrity of the normal synaptic membrane.

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**TABLE I**

<table>
<thead>
<tr>
<th>Addition</th>
<th>[Ca^{2+}] (nM)</th>
<th>Dye leakage (%)</th>
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</thead>
<tbody>
<tr>
<td>None</td>
<td>334 ± 14</td>
<td>19.2 ± 1.0</td>
</tr>
<tr>
<td>Chlordecone</td>
<td>745 ± 152</td>
<td>60.8 ± 6.7</td>
</tr>
<tr>
<td>Chlordecone + ganglioside GD1A</td>
<td>446 ± 44</td>
<td>61.8 ± 5.0</td>
</tr>
<tr>
<td>Ganglioside GD1A</td>
<td>350 ± 10</td>
<td>41.2 ± 2.1</td>
</tr>
</tbody>
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roviscosity [9]. A relatively minor change in microviscosity can have major effects on the permeability of a membrane to ions [20]. Such bidirectional potential of ganglioside-related effects depending on whether or not exogenous agents are present, is paralleled by the data on $[\text{Ca}^{2+}]_i$ levels of synaptosomes presented here.

The mechanism underlying the apparently trophic phenomena enabled by gangliosides [19, 25] is unclear. The various possibilities that have been proposed include stimulation of adenylate cyclase activity [21], which can enhance neurite formation in culture [22], activation of the sodium pump [17], stimulation of synthesis of some membrane constituents [13] and modulation of protein phosphorylation [2]. Within the brain gangliosides are largely confined to neurons and 12% of it is present in the membranes of nerve terminals [16].

Our results suggest that the protective effect on neurons, reported for $\text{GM}_1$ may be by way of an enhancement of physical properties critical for membrane stability. These may include both decreasing the permeability of the plasma membrane to calcium ions and increasing the resistance of synaptosomes to lysis subsequent to chemically induced membrane damage.

This study was supported by National Institute of Environmental Health Grant ES 04071


26 Walsh, T.J., Emerich, D. and Schmeckel, D., GM₁ ganglioside attenuates the behavioral deficits but not the granule cell damage produced by intrahippocampal colchicine, Brain Res., in press.