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Journal

Neuroscience letters, 84(2)

ISSN

0304-3940

Authors

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Publication Date

1988

DOI

10.1016/0304-3940(88)90413-2

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Peer reviewed

NSL 05070

GM₁ 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 GM₁, 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 GM₁ 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 GM₁ 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 [Ca²⁺]_i within the surviving synaptosomes [5]. Under such circumstances, pretreatment with GM₁ 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].

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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 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), $R_{\min}$ (the ratio of fluorescence at 340 nm/380 nm in the absence of Ca^{2+}) and $R_{\max}$ (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 $19.5 \pm 0.9\%$ ($n=34$). Data are the means of determinations on 5 separate rat brains. Bars represent S.E.M.

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 $[Ca^{2+}]_i$ in synaptosomes significantly by 33.2 ± 11 nM ($n=24$, $P<0.01$), when present at 50 μ M 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 $[Ca^{2+}]_i$ in synaptosomes exposed to GM₁. GM₁ also caused a very minor increase in the leakage of fura-2 out of synaptosomes, by $3.2 \pm 1.7\%$ ($n=19$).

A parallel study using the disialoganglioside GD_{1A} was also carried out. This ganglioside mitigated the effect of chlordecone upon synaptosomal $[Ca^{2+}]_i$ but the increased leakage of dye caused by chlordecone, was not protected against (Table I). In fact, GD_{1A} alone caused a major elevation of fura-2 leakage (Table I). Thus the effects on $[Ca^{2+}]_i$ 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 GD_{1A} 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 GD_{1A} 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 mic-

TABLE I

EFFECT OF CHLORDECONE IN THE PRESENCE AND ABSENCE OF GANGLIOSIDE GD_{1A} UPON SYNAPTOSOMAL INTEGRITY

Data represent the mean from synaptosomes of 5 separate rats. Incubation with or without 50 μ M chlordecone was for 15 min at 37°C and in some cases this was preceded by a 5-min incubation with 50 μ M GD_{1A}.

Addition	$[Ca^{2+}]_i$ (nM)	Dye leakage (%)
None	334 ± 14	19.2 ± 1.0
Chlordecone	745 ± 152	60.8 ± 6.7
Chlordecone + ganglioside GD _{1A}	446 ± 44	61.8 ± 5.0
Ganglioside GD _{1A}	350 ± 10	41.2 ± 2.1

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 $[Ca^{2+}]_i$ 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 GM₁ 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

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