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SOME FUNCTIONAL CONSEQUENCES OF CHRONIC GM_1 GANGLIOSIDE ADMINISTRATION IN BRAIN DAMAGED RATS

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Abstract. Systemic injections of GM_1 gangliosides can enhance behavioral recovery from brain damage as measured by a number of cognitive tasks. The functional recovery is not due to GM_1-induced alterations in activity, emotional arousal, or heightened sensitivity to mild, noxious stimulation. In addition, the recovery endures long after all treatments are terminated. Although the specific actions by which GM_1 treatments facilitate recovery are unknown, evidence does suggest that both anomalous sprouting and protection of neurons from secondary consequences of injury may be involved in the repair process.

Now that there is sufficient evidence to demonstrate that recovery from CNS trauma is a reproducible phenomenon, there is a renewed effort to find systemically administered agents which can cross the blood-brain barrier and enhance both the rate and final outcome of brain injury repair. Pharmacological treatment for brain damage is a relatively new development in neurology. Ten years ago, for example, one clinician could write:

> "Clinicians concerned with the physical therapy and general rehabilitation of persons with incapacitating brain lesions rarely make use of drugs to alter the course of the disease, and in general, the pharmacological management of these patients is only symptomatic (Brailowsky, 1980, p. 187)".

Norman Geschwind (7), a distinguished neurologist concerned with language disorders following brain injury, also expressed concern about the lack of any real therapy. He noted that:

> "...there must be many cases in which the capacity for recovery is latent, and revealed only by some further manipulation, but experimenters have only rarely been zealous in their search for the right maneuver" (p. 1).

The advantages of finding a systemic treatment for brain injury are obvious: (1) treatments can be given "as needed" with little, if any, distress to the patient; (2) systemic administration (oral or by injection) is safer and much less costly than treatment by chronic, intracerebral infusion of substances such as NGF (15) or the grafting of embryonic brain tissue into the damaged host brain; (3) non-surgical interventions can be administered much more rapidly (e.g. at home or at the site of a traumatic accident) thus minimizing the effects of the trauma.

One agent meeting the above criteria that we have used successfully in our laboratory is GM_1 ganglioside. We became interested in the potential, therapeutic effects of GM_1 treatments following a report by Barbara Oderfeld-Nowak (17), who was one of the first to show that ganglioside injections could enhance ChAT and AChE activity after septo-hippocampal lesions had disrupted the cholinergic pathways in adult rats. Shortly thereafter, Karpiak (10) demonstrated that rats who were treated with gangliosides immediately prior to, and then following unilateral lesions of the entorhinal cortex, made less errors that untreated counterparts when they were tested for retention of a previously learned spatial alternation task. In a follow-up study, Ramirez et al. (19) were able to show that chronic GM_1 injections could also facilitate behavioral recovery after bilateral, entorhinal cortex damage.

At the time our laboratory began to investigate ganglioside-induced functional recovery, there was very little evidence to demonstrate that the treatments could also affect *acquisition* of a cognitive task; that is, if the CNS injuries were suffered *before* specific learning of a complex nature, and if all treatments were begun after bilateral damage had been inflicted. In our first experiment (24), we used electrocoagulation to create large, bilateral lesions of the caudate nucleus in 24 adult rats. Immediately after the surgery the rats were given a series of 14, intraperitoneal injections of GM_1 gangliosides. Nine days after the surgery the animals began testing on a shock-avoidance, spatial reversal task.

In this situation rats are first taught to run to one side of a two-choice maze in order to avoid or escape from foot-shock. Once this is learned, the animals are then required to run to the previously shocked side to avoid or escape the negative reinforcement (i.e. reverse the previously 'correct' behavior). Normal animals can learn to shift responses relatively easily, but those with caudate nucleus damage will perseverate. This means that the brain-injured rats have considerable difficulty in giving up the previously learned response even though it is no longer adaptive. To test for recovery, we gave the animals a continuous series of spatial reversal trials for 30 days (10 trials/day). We then gave the animals a 'rest period' and then retested them two months later to determine if there were long-lasting, beneficial effects of the ganglioside treatments.

Our findings revealed that, in comparison to untreated rats with severe, caudate nucleus injury, those given chronic ganglioside injections, fared significantly better on a number of behavioral measures. For example, in comparison to their brain-injured counterparts given isotonic saline injections, GM_1-treated rats reached the goal area more often per reversal, took fewer days to reach criterion after the first reversal and reached criterion (nine out of ten successively correct trials) more often. It is important to note that the behavioral differences between the GM_1-treated and brain-injured controls was noticeable within the first 10 days of training and the differences remained throughout the 30 days of testing. We noted that, despite their improved performance relative to the non-treated group, the rats treated with GM_1 did not perform as well as the normal animals.

On the retest of the task 2 months later, all animals were able to show retention of what they had originally learned. Here, the ganglioside treated rats no longer differed from intact controls, although the brain-damage, saline-injected rats still displayed a learning impairment.

Although the results demonstrated a beneficial effect of chronic ganglioside treatments, from a strictly behavioral perspective, one might argue that the treatments might have improved performance not so much by affecting brain repair *per se*, but perhaps by having an antinociceptive influence (10), or by heightening motor activity or arousal such as might be expected after treatments with amphetamine (6). A new study would also give us: (1) the opportunity replicate our earlier findings; (2) compare ganglioside treated rats to those given systemic injections of saline, on open field activity and shock sensitivity. The procedures for surgery and testing were identical to those used by Sabel, Slavin and Stein (23), except that rats began testing only five days after surgery rather than nine (see 4 for specific details). On the total number of avoidances in the initial stages of learning, ganglioside-treated rats did significantly better that the lesion control group, although they were impaired with respect to intact animals. The rats given ganglioside also made more escapes, had fewer perseverative errors and fewer failures per reversal than lesion-alone counteparts, thus demonstrating again that chronic, systemic administration of this substance could enhance functional recovery of cognitive performance after severe brain injury.

Next, we explored the question of whether ganglioside treatments would affect locomotor activity and sensitivity to shock. Five days after receiving bilateral caudate nucleus lesions rats received chronic saline or ganglioside injections and were then subjected to 5 days of open field testing followed by an assessment of foot-shock sensitivity.

Analysis of open field activity scores revealed no differences in activity levels or location preferences. Also, in comparison to intact controls, treated and untreated animals crossed the same number of lines and grids and hugged the walls in the same manner. With respect to shock sensitivity, no differences among the groups were observed.

In general, our findings reveal that systemic injections of gangliosides do seem to have direct effects on restoration of behavioral functions after brain trauma. As Karpiak, et al. (11) also observed, ganglioside effects can be seen within 48 h after treatment begins. Although amphetamine treatments in rats with caudate lesions (4) can also produce similar, beneficial effects after injury, the recovery takes longer to occur (up to 10 days later) and the performance of the animals is not as consistent as the effects obtained with ganglioside treatments.

The fact that the ganglioside-treated rats did not differ from untreated, brain injured counterparts on open-field activity or sensitivity to shock, can be taken to suggest that the effect of the drug on facilitating cognitive recovery is not simply due to increased arousal or enhanced locomotor activity. Although gangliosides have been applied primarily as treatment for CNS *injury,* the substance may have nootropic properties which need to be better defined.

With respect to the molecular and physiologic properties of gangliosides, much more is known and there are numerous reference sources available (e.g., 12, 18, 20, 25, 26). In our laboratory, we have been concerned with the structural and anatomical changes induced by ganglioside treatments, rather than its ability to affect molecular and metabolic alterations. Although there is a very significant literature on the biochemistry of gangliosides there is also mounting evidence to demonstrate that these substances also seem to play a role in regulating neuronal cell growth, adhesion and synaptogenesis (8, 9, 21, 27). In thinking about our behavioral results, we reasoned that the enhanced recovery could be due to the ganglioside's effect on rescuing neurons from anterograde and/or retrograde degeneration, or the enhancement of collateral sprouting following the significant, neuronal denervation resulting from the injury. We tested this hypothesis by studying the effects of chronic ganglioside injections in adult rats that had received massive, unilateral transections of the nigrostriatal pathways (23). Rats with almost complete transections of the nigrostriatal system will show a stereotyped, ipsiversive rotation which eventually disappears, but which can be strongly reinstated by injection of either amphetamine or apomorphine.

First, as Toffano and his colleagues (27) had previously shown, we found that ganglioside-treated rats rotated significantly less than brain damaged conspecifics given saline injections. We hypothesized that the beneficial effects of the treatments were due either to the preservation of nigrostriatal fibers on the same side as the lesion, or to the reinnervation of the striatum from neurons providing collateral 'sprouts' from the contralateral substantia nigra.

Accordingly, we injected wheat-germ agglutinated horseradish peroxidase into the denervated caudate nucleus in order to study both retrograde and anterograde transport of the enzyme back into neurons of the ipsilateral and contralateral substantia nigra and ventral tegmentum. We were then able to count the cell bodies of neurons containing the HRP label. Within three days after surgery, there was a major loss of both ipsi- and contralateral connections to the caudate in both treated and non-treated rats. By 15 days after injury, however, the rats given GM_1 had significantly more labeled neurons both ipsilateral and contralateral to the hemitransection. More interestingly, the number of labeled neurons that picked up the HRP in the contralateral substantia nigra was significantly higher than normal. This finding can be taken to indicate that neurons in the contralateral SN which do not normally send projections across the midline, sprout new collaterals which then grow across to innervate the damaged striatum.

In a second morphological experiment, Sabel et al. (22), used the Fink-Heimer silver degeneration stain to determine whether newly sprouted fibers following ganglioside treatments could innervate a damaged structure. We reasoned that, if gangliosides do cause increased terminal sprouting and terminal proliferation, then a 'secondary' lesion would result in greater terminal degeneration than if the synaptic terminal population were of 'normal' density.

Thus, groups of rats were first given unilateral hemitransections followed by chronic treatments with gangliosides or saline. Fifteen days after the hemitransection surgery, a neurotoxin (6-OHDA) was injected into the ipsilateral substantia nigra or into the ipsilateral ventral tegmentum. Nine days after this surgery the animals were killed for histological examination using the Fink-Heimer stain.

Briefly stated, the animals treated with gangliosides had significantly more (approximately 60%) degenerating terminals in the caudate than lesion rats given saline injections. These data seem to argue strongly for an enhanced ***structural and morphological*** basis for the functional recovery produced by ganglioside treatments. The appearance of more degenerating terminals cannot be attributed easily to ganglioside-induced, enhanced axonal transport of enzymes or proteins, although that may very well be another, potential recovery mechanism. Recent work of Jackson, Jenner and Marsdon (9), provide confirming evidence for ganglioside induced, morphological plasticity. These investigators made unilateral, electrolytic lesions of the substantia nigra and then studied apomorphine-induced rotational behavior in GM_1-treated and saline controls.

As we would have predicted, the GM_1 treatments reduced rotational activity and, at the morphological level, caused a reduction in glial scarring. Using Tyrosine-hydroxylase immunocytochemistry, Jackson and colleagues observed more labeled neurons in the damaged SN than in untreated counterparts. Taken together with our findings, these data strongly suggest that GM_1 treatments can reduce the severity of brain injury at both the behavioral and morphological levels. Recently (13) we have examined this question of ganglioside-induced, neuronal sparing using a different brain damage model. We destroyed the nucleus basalis magnocellularis in adult rats by injection of ibotenic acid directly into the nucleus. Immediately after this surgery animals received chronic injections of saline or GM_1 for 14 days and were then allowed to survive for 2 months. Since nucleus basalis lesions deplete the cortex of most of its cholinergic input, AChE staining was used as an indicator of treatment effects.

Our results showed that GM_1 treated rats given ibotenic acid lesions of NBM, showed more rapid recovery of passive avoidance learning, had a lower density of AChE staining in the cortex, fewer glia and less ventricular swelling than saline- treated counter-parts. In normal rats treated with GM_1, we also observed less AChE staining than rats given saline. Although these data could be taken to suggest that the GM_1 was causing some type of neuronal loss or some potentially toxic effect, further investigation led to an entirely different conclusion. Both cresyl violet (used to count surviving neurons in NBM) and immunocytochemical staining for ChAT (to detect levels of the enzyme that produces ACh) revealed no differences between treated and untreated normal animals. This observation suggests that ACh itself was not being reduced by the ganglioside injections since ChAT staining was the same as for saline controls; however, it *is* evidence that the normal metabolic activity of neurons may be modified by chronic ganglioside treatments.

Our observation that, in normal animals, AChE is decreased by ganglioside treatments, could be taken as evidence that GM_1 may have nootropic effects. The ganglioside-induced decrease of AChE the enzyme which degrades ACh, could lead to enhanced learning and memory in normal subjects. There are recent data demonstrating that physostigmine (5, 16), a cholinergic stimulant, can be used to facilitate learning in normal animals. Decreasing AChE levels at the synapse might have similar effects, since more ACh would be available (or would act for a longer period of time) to affect cholinergic neurons. Other evidence suggests that cholinergic antagonists can block memory consolidation and learning (1). Recently, Di Patre et al. (3), used intraventricular administration of vincristine to damage the cerebral cholinergic system in adult rats. These workers found that the alkaloid causes a 45% decrease in hippocampal ChAT activity and a 35% decrease in high affinity choline uptake. Chronic GM_1 injections prevented the decrease in ChAT activity and blocked the decline in HACU uptake. Motor deficits (ability to remain on a small, elevated platform and failure to grasp a wire for support) were eliminated by the GM_1 treatments. Apparently, the treatments blocked the degeneration of the septo-hippocampal cholinergic fibers caused by the vincristine injections. GM_1 treatments were also used specifically by Mahadik et al. (14) to block some of the neurotoxicity caused by ibotenic acid injections into the nucleus basalis. These investigators noted that chronic GM_1 treatments significantly reduced the loss of animals that typically accompany ibotenic acid lesions and reduced the decline of both ChAT activity and AChE. Thus, there seems to be growing evidence that gangliosides may mediate their effects on restoration of behavioral functions through a number of different pathways — as the proceedings of this conference have demonstrated.

In conclusion, chronic ganglioside injections in brain damaged adult animals have been shown to produce reliable, behavioral recovery of complex learning in a number of test situations. Although the specific neuronal mechanisms by which gangliosides exert their effects are still being debated and defined, it now appears that this substance may be an effective treatment for certain types of traumatic brain damage. Whether the ganglioside treatments can work alone to produce neuronal repair, or would be more effective when provided in combination with other trophic substances, awaits more confirmation. The beneficial effects of ganglioside treatments in traumatic brain injury repair will also need to be tested in careful clinical models designed to replicate the findings obtained in the animal laboratory setting where injuries and experimental conditions are much more easily defined and manipulated.

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