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Effects of Intraventricularly Applied Gangliosides and N-Acetylneuraminic Acid on Acquisition and Retention Performance of a Brightness Discrimination Task in Rats

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POPOV, N., G. TOFFANO, U. RIECHERT AND H. MATTHIES. *Effects of intraventricularly applied gangliosides and N-acetylneuraminic acid on acquisition and retention performance of a brightness discrimination task in rats.* PHARMACOL BIOCHEM BEHAV **34**(2) 209–212, 1989.—The effects of intraventricularly applied gangliosides (about 90 to 120 nmoles/10 μl) and N-acetylneuraminic acid (800 nmoles/10 μl) on acquisition and retention of a brightness discrimination task were studied in rats. Single injections of GT_{1b} and N-acetylneuraminic acid improved the retention performance. GM_1 showed an enhancing effect on both acquisition and retention, while GD_{1a} and GD_{1b} exerted no influence on the behavioral parameters tested. The findings presented show how memory may be improved by exogenous application of substances commonly occurring in the mammalian body.

Learning and memory Brightness discrimination Exogenous gangliosides N-acetylneuraminic acid Rats

NUMEROUS investigations have indicated the involvement of glycomacromolecules such as glycoproteins and gangliosides in various CNS processes (2, 6, 18, 21, 23, 25) and, especially, in neurobiological events which mediate or modulate the molecular coding of acquired information associated with adaptive functions, neuronal plasticity and even the formation of memory [see (1, 12, 13, 16, 17, 19, 22, 24, 26) for review]. Since the role of glycomacromolecules in learning and memory processes is not yet fully understood, we investigated several carbohydrate components of glycoproteins in suitable behavioral models in rats. Thus, L-fucose and N-acetylneuraminic acid (NANA) applied intraventricularly or intraperitoneally by a single injection before training in shuttle-box avoidance as well as in shock-motivated brightness discrimination in rats, significantly improved the retention of an acquired behavior as measured 24 hr later (15,26).

The effect of NANA in improving the retention performance of an acquired behavior is consistent with findings demonstrating the inhibitory action of intraventricularly applied antibodies to the monosialoganglioside, GM_1, on retention performance of a learned avoidance response (9,10). It has also been shown that chronic systemic treatment with definite ganglioside mixture or total ganglioside extracts improved learning and memory in various animal models (5, 8, 11).

These literature reports, along with our previous results obtained by NANA application, prompted us to study whether intraventricularly injected individual ganglioside species (GM_1, GD_{1a}, GD_{1b} and GT_{1b}) and NANA at appropriate single doses may influence memory parameters of the brightness discrimination task usually employed in our laboratory (14).

METHOD

Male Wistar rats weighing approximately 200 g were used throughout. The animals were provided with food and water ad lib.

The four highly purified gangliosides (GM_1, GD_{1a}, GD_{1b} and GT_{1b}) were produced by Fidia Research Laboratories, Abano Terme/Padova, Italy, while NANA was from Serva Fine Biochemicals, Heidelberg, F.R.G. The chemicals were dissolved in physiological saline, and in each case 10 μl was intraventricularly injected through an implanted cannula using a Hamilton microsyringe. The following stereotaxic coordinates were used: 0.25 mm AP of the bregma, 1.6 mm lateral of the mediane suture, 3.5 mm deep beneath the skull into the right lateral ventricle; the correct positioning of the inserted cannula was verified histologically. Using an experimental model of associative learning (brightness discrimination), preliminary tests revealed that GM_1 was effective at a single dose of 200 μg/10 μl. Hence, all gangliosides were

TABLE 1

EFFECTS OF GANGLIOSIDES ON ACQUISITION OF A BRIGHTNESS DISCRIMINATION TASK IN RATS

Treatment	Correct Responses	Errors	Reaction Time (sec)	Run time (sec)
Saline	20.2 ± 1.0	10.8 ± 1.0	1.7 ± 0.3	3.6 ± 0.3
GM_1	23.3 ± 0.9*	7.7 ± 0.9*	2.5 ± 0.3	3.6 ± 0.3
GD_{1a}	20.5 ± 1.1	10.5 ± 1.1	2.1 ± 0.4	4.4 ± 0.5
GD_{1b}	22.3 ± 0.6	8.7 ± 0.6	1.4 ± 0.2	3.6 ± 0.4
GT_{1b}	20.4 ± 1.0	10.6 ± 1.0	1.3 ± 0.3	4.2 ± 0.8

All gangliosides were intraventricularly injected (200 μg/10 μl) one hour before training. All values are means ± S.E.M. (n = 8 animals in each group) and where significantly different from the saline controls ($p<0.05$) are indicated by an asterisk. See text for further details.

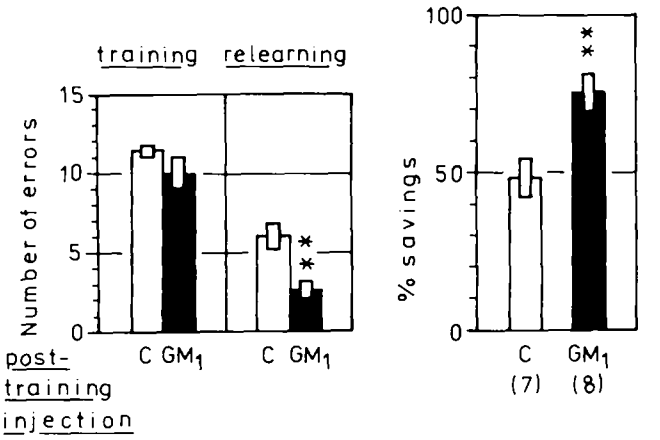

FIG. 1. Retention-improving effect of GM_1. The ganglioside was intraventricularly injected (200 μg/10 μl) into rats immediately upon completion of training on the brightness discrimination reaction, the relearning test being performed 24 hours after starting training. Means ± S.E.M.; number of animals in parentheses. **Indicates $p<0.01$, GM_1-treated animal vs. saline controls (C).

given at a dose of 200 μg/10 μl, the molar doses ranging from 90 to 120 nmoles/10 μl due to the different NANA amounts in the individual gangliosides. NANA was injected at 800 nmoles/10 μl, a dose which had previously been reported to be effective (15).

The rats were assigned to one of four experimental groups depending on the time of ganglioside or NANA injection before starting the behavioral test (i.e., before training) or whether it was injected immediately upon completion of training:

Series No. 1: Injection of all four gangliosides one hour before training.

Series No. 2: GM_1 injection immediately upon completion of training.

Series No. 3: NANA injection 30 min before training.

Series No. 4: GM_1 and NANA each applied one or four hours before training.

It should be noted that in no case did the animals show any toxic or side effects as a consequence of this treatment.

The rats were trained to learn foot-shock-motivated brightness discrimination in a semiautomatic Y-maze using the behavioral model of associative learning described by Ott and Matthies (14). Briefly, the learning task consisted of escaping from a mild electric shock applied to the grid floor of the starting compartment, into the illuminated alley of the Y-maze, i.e., the target compartment. Reaction time and run time were automatically registered. This is contrary to a rat's innate reaction to escape into the dark. Runs into the nonilluminated alley were punished by application of 1-mA current to the grid floor of the dark alley of the maze, whereas the illuminated alley was the safe area. The side of illumination was changed after every three trials (runs) in order to avoid positional training. The runs were counted as correct responses only if the animals entered the illuminated alley of the maze. The mean intertrial interval was 1 min. The training session was completed after 31 trials. Retention of the brightness discrimination reaction was measured in a relearning session 24 hr after training.

Evaluation of Results

Running into the nonilluminated alley of the Y-maze was scored as an error. To express the retention performance (retention index), the number of training errors and the number of relearning errors were used to calculate the percent savings exhibiting a value of approximately 50% for controls receiving 10 μl of physiological saline:

$$\% \text{ savings} = \frac{\text{training errors minus relearning errors}}{\text{training errors}} \cdot 100$$

TABLE 2

EFFECTS OF GANGLIOSIDES ON RETENTION PERFORMANCE (RELEARNING PROCEDURE) OF A BRIGHTNESS DISCRIMINATION TASK IN RATS

Treatment	Correct Response	Errors	Retention Index (% savings)	Reaction Time (sec)	Run Time (sec)
Saline	24.4 ± 0.7	5.6 ± 0.7	49.1 ± 4.5	1.3 ± 0.2	2.6 ± 0.3
GM_1	28.0 ± 0.5*	3.0 ± 0.5*	64.8 ± 3.6†	1.8 ± 0.3	2.6 ± 0.4
GD_{1a}	25.9 ± 0.7	5.1 ± 0.7	51.6 ± 6.6	1.5 ± 0.4	2.8 ± 0.8
GD_{1b}	25.5 ± 0.7	6.5 ± 0.7	40.4 ± 6.4	1.2 ± 0.3	2.5 ± 0.4
GT_{1b}	27.5 ± 0.5	4.0 ± 0.5	62.2 ± 3.5†	0.9 ± 0.1	2.1 ± 0.2

All gangliosides were intraventricularly injected (200 μg/10 μl) one hour before training, the relearning test being performed 24 hours after starting training. All values are means ± S.E.M. (n = 8 animals in each group) and where significantly different from the saline controls, *$p<0.05$ and †$p<0.01$, respectively. See text for further details.

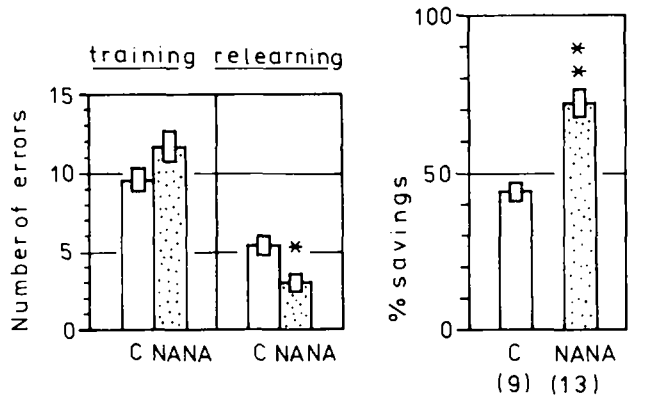

FIG. 2. Retention-improving effect of 0.8 μmoles N-acetylneuraminic acid (NANA) injected intraventricularly 30 min before training, the relearning test being performed 24 hours after starting training. Means ± S.E.M.; number of animals in parentheses. *Indicates $p<0.05$, **stands for $p<0.01$, NANA-treated animals vs. saline controls (C).

clarify the ineffectiveness of GD_{1a} at the dose used. The trisialoganglioside, GT_{1b}, was effective in terms of improving retention performance of the acquired behavior (Table 2).

Series No. 2

In order to elucidate the effect exerted by GM_1, this monosialoganglioside was intraventricularly injected in another series (designated as No. 2 series) immediately upon completion of training. As shown in Fig. 1, GM_1 caused a marked improvement of retention performance. Thus, the results of both experimental series No. 1 and No. 2 showed that GM_1 improved acquisition and retention performance of the acquired behavior. This implies that GM_1 may be involved in different biochemical mechanisms operating during the early (acquisition phase) and the late (memory formation phase) stages of consolidation of the memory trace (12,16).

Series No. 3

NANA applied intraventricularly 30 min before training gave

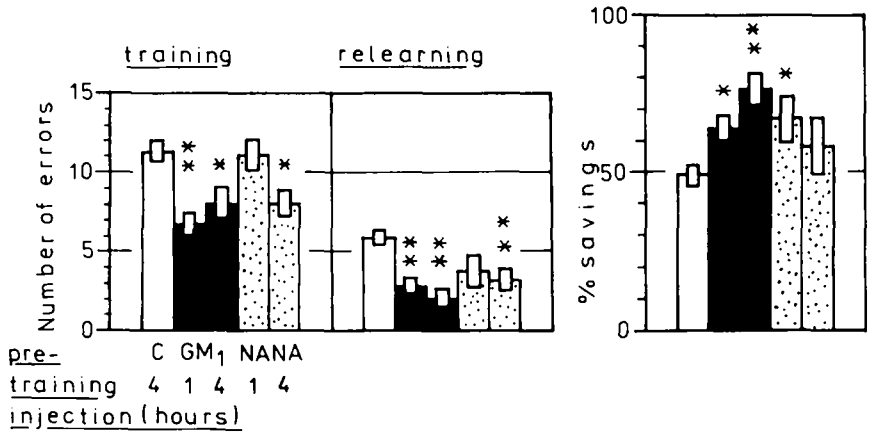

FIG. 3. Effects of GM_1 (200 μg≅0.09 μmoles/10 μl) and NANA (0.8 μmoles/10 μl) intraventricularly applied one or four hours before training, the relearning test being performed 24 hours after starting training. All values are means ± S.E.M. (n = 6–10) and, where significantly different from the saline controls (C), are indicated by one and two asterisks for $p<0.05$ and $p<0.01$, respectively.

Statistics

The two-tailed Student's *t*-test was used to compute the statistical significance of differences between means of ganglioside or NANA-treated animals vs. saline controls.

RESULTS AND DISCUSSION

Series No. 1

Tables 1 and 2 summarize the results obtained in the series in which the gangliosides were intraventricularly injected one hour prior to the training session. As shown in the tables, the ganglioside treatment did not affect the behavior of the experimental animals in terms of reaction time or run time. Under the conditions used, GM_1 improved both learning performance (acquisition) and retention performance (memory formation) in the behavioral test. Under the same experimental conditions GD_{1a} and GD_{1b} had no effect, although an effect, at least of GD_{1a} (catabolic precursor of GM_1), had been expected. Additional investigation is required to an improvement of retention, as described elsewhere (15). The present results are depicted in Fig. 2. Dosage comparison between NANA and GM_1 unequivocally demonstrated the substantially more potent action of GM_1 (about 120 nmoles/10 μl containing approximately 19% NANA in its molecule) than of NANA per se (800 nmoles/10 μl). In view of the extra availability of NANA needed to produce the retention-improving effect of GM_1 [NANA doses lower than 500 nmoles/10 μl were ineffective (15)], there is no reason to assume that the effect of exogenous GM_1 was associated only with supply of NANA to meet higher demands under altered functional conditions. However, the significance of the rate of reutilization of the NANA moiety of exogenous GM_1 (4) cannot be assessed.

Series No. 4

Another experimental series compared the effective doses of GM_1 and NANA with these substances being applied one or four hours prior to the training session. Despite the different effect of

NANA on training and relearning, depending on pretreatment time, the retention index (based on % savings) showed that the NANA-induced improvement of retention performance occurred only when this substance was applied one hour before training (Fig. 3). On the other hand, GM_1 applied 4 hours prior to training showed an even more marked effect on retention performance. However, under these conditions, it cannot be distinguished which effect, that on acquisition or retention, or on both, prevailed.

To summarize, the present study of the effects of the exogenous gangliosides, GM_1, GD_{1a}, GD_{1b} and GT_{1b}, on an acquired behavior showed GM_1 to have a potent improving effect on both acquisition and memory formation, while GT_{1b} effected memory formation only. The ineffectiveness of GD_{1a} and GD_{1b} cannot be easily interpreted. One possibility of many is that the proposed conversion of mainly GD_{1a} into GM_1 (3, 4, 21) occurred only to a limited extent and, hence, was not sufficient to exert an effect. Another explanation could be that GD_{1a} and GD_{1b} were not all converted into other gangliosides during the time of the experiment and, thus, were ineffective per se.

Despite difficulties in interpreting these findings the present work is a first attempt to study the acute action of exogenous gangliosides on acquisition and retention of an acquired behavior. Investigating the exact cellular mechanisms of action of exogenous gangliosides in learning and memory processes as a form of neuronal plasticity is an intriguing goal for future work.

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