Pharmacology Biochemistry & Behavior, Vol. 39, pp. 825–828. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

BRIEF COMMUNICATION

Gangliosides Improve a Memory Deficit in Pentylenetetrazol-Kindled Rats

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Received 4 September 1990

GRECKSCH, G., A. BECKER, C. GADAU AND H. MATTHIES. *Gangliosides improve a memory deficit in pentylenetetrazol-kindled rats.* PHARMACOL BIOCHEM BEHAV **39**(3) 825–828, 1991.—Epileptic patients often show impairments in a number of cognitive functions. Kindling is considered to be a useful experimental model for human epilepsy. Recently we have demonstrated a learning impairment in a shuttle box experiment in pentylenetetrazol (PTZ)-kindled rats. This model offers the possibility to investigate the relation between repeated convulsions and their consequences on learning and on the other side to test the effectiveness of substances on both processes. Although systemic application of gangliosides has neither an effect on the development of seizures induced by repeated injections of PTZ, nor on seizures induced by PTZ in kindled animals, the treatment protects against the memory-impairing effect of convulsions. These findings suggest a new useful strategy in the therapy of epileptic patients with the aim of diminishing the psychosocial problems in persons with seizure disorders: a combination of the anticonvulsive basic therapy and gangliosides.

Epilepsy Kindling PTZ Learning Shuttle box Gangliosides Rats

CLINICAL observations show that psychosocial problems are prevalent in persons with seizure disorders and that epileptic patients often are faced with memory deficits and impairments in a number of cognitive functions (4,11). Kindling has become a widely employed technique for studying seizure mechanisms and is considered to be a useful experimental model for human epilepsy. Kindling is induced by repeated exposure to electrical stimuli (6) or repeated administration of convulsant drugs (15) resulting in an increased responsiveness to the stimulus culminating in a generalized convulsion. Recently, impairments of cognitive processes in kindled animals were described (12, 21, 24). We could demonstrate a clear impairment of active avoidance learning in a two-way shuttle box in pentylenetetrazol (PTZ)-kindled rats (2). This model allows a test of the ability of different drugs to improve the learning deficit related to repeated convulsions. In this regard gangliosides, a family of sialic acid-containing glycosphingolipids particularly abundant in brain tissue seem to be of special interest. They are well known constituents of cellular membranes. The integrative function of these complex glycolipids in the brain are not clearly understood but some properties are well established. Exogenous gangliosides promote structural repair after brain lesions in vivo (20,23), which may have implications for recovery of function (5). This facilitated recovery may be due as well to a prevention of degeneration and a subsequent neuronal regeneration or both together (9). The protecting effects of gangliosides were used in the clinical therapy of different neurological disorders and disturbances.

We tested the effectiveness of exogenous gangliosides to improve the kindling induced learning deficit using applications of the substance as well as in the course of kindling development and during the learning experiment.

METHOD

Animals

Experiments were performed with 124 adult male Wistar rats from our own breeding stock. The animals were kept under controlled laboratory conditions under a lighting regime of LD 12:12 (light on at 6:00 a.m.), temperature 20 ± 2°C, and relative air humidity 55–60%. They had free access to commercial rat pellets and tap water. The rats were housed in groups of 6–8.

Kindling Procedure

For PTZ kindling an initially subconvulsive dose of 45 mg/kg body weight PTZ was injected intraperitoneally (IP) once every 48 h. After each injection the convulsive behavior was observed for 20 min. The resultant seizures were classified as follows: stage 0: no response; stage 1: ear and facial twitching; stage 2: convulsive waves through the body; stage 3: myoclonic jerks, rearing; stage 4: turn over into side position; stage 5: turn over into back position, generalized clonic-tonic seizures. The dura-

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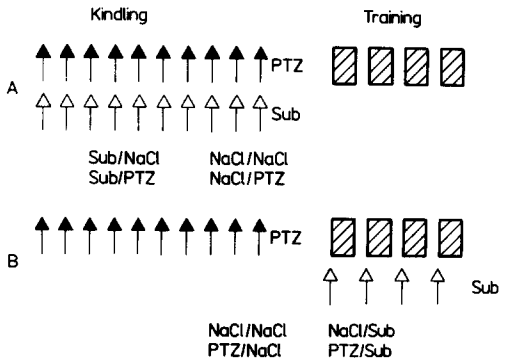

FIG. 1. Experimental schedule. Black arrows: injections of 45 mg/kg PTZ IP; White arrows: injections of 10 mg/kg gangliosides (Sub) IP. Rectangles: daily training sessions in a shuttle-box 20 trials/day.

tion of the PTZ convulsions was not registered.

Control animals received the same number of saline injections.

Learning Procedure

Two-way active avoidance-shuttle-box. The automatic shuttle-box was divided into two compartments $0.25 \times 0.25 \times 0.6$ m separated by a 5 cm hurdle. The conditioned stimuli were 40-W bulbs located on the central ceiling of each compartment and a sound produced by a buzzer. The unconditioned stimulus was an electric foot-shock of 1 mA delivered through stainless rods forming the floor. The conditioned stimuli/unconditioned stimulus interval lasted 4 s. One trial was limited to 20 s. Each session consisted of 20 trials and was repeated on four consecutive days.

Sessions were performed during the light part of the 12:12 h cycles at about the same time ± 1 h. Prior to the first session, the rats were allowed to explore the box for 5 min, and on the following days one min was provided.

Experimental Design

The rats were considered to be kindled after receiving 10 PTZ injections. Kindled and control animals were tested in the learning experiment 24 hours after the last injection.

The effect of systemically administered gangliosides on the learning deficit was investigated in two different experiments (A and B in Fig. 1): A) The gangliosides or saline were injected IP in a dose of 10 mg/kg 1 h before each PTZ and NaCl injection, respectively. The learning experiment was performed without any injections. B) The animals were kindled. Gangliosides were injected during the learning experiment, 1 hour before each daily training session.

In a third experiment the effect of 10 mg/kg gangliosides on the convulsive effect of a challenge dose of 45 mg/kg PTZ IP was tested using fully kindled rats. Gangliosides were IP injected 1 h before PTZ.

The gangliosides used in this study were produced in the Institute of Pathobiochemistry of the Medical Academy Magdeburg from pig brain with the following pattern: GQ1b = 4.4%, GT1b = 26.8%, FucGD1b = 6.5%, GD1b = 13.4%, GD1a = 38.6%, GD3 = 5.3% and GM1 = 5.0% Svennerholm nomenclature (22). The content of neuraminic acid was about 18%. The purity of the ganglioside mixture was about 98%.

Statistics

Statistical evaluation was performed using the two-tailed Mann-Whitney U-test.

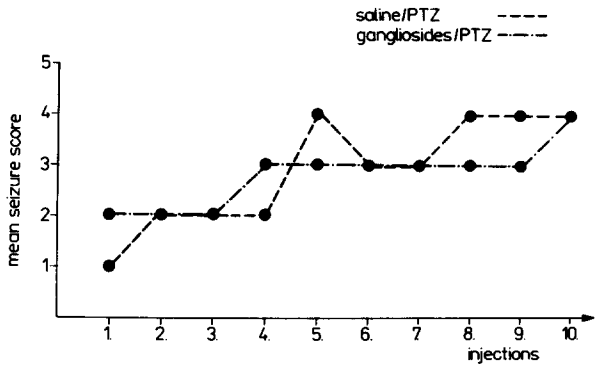

FIG. 2. Effect of gangliosides on the development of kindling.

RESULTS

If the gangliosides were injected during the course of kindling development they show no clear effect on the resulting seizures (Fig. 2). Nevertheless, the gangliosides exert an improving effect on the PTZ kindling induced impairment in the shuttle-box learning (Fig. 3). On the other hand the gangliosides do not improve the learning performance of control animals under these experimental conditions.

In the second experiment we tested the effect of gangliosides on fully kindled animals. Also under these conditions the gangliosides were able to normalize the learning performance of PTZ kindled animals (Fig. 4). In contrast, an acute administration of gangliosides has no effect on the convulsions induced by a challenge dose of PTZ in fully kindled rats (Fig. 5).

DISCUSSION

Our results demonstrate a clear improving effect of systemically applied gangliosides on a learning deficit in kindled rats. In contrast the gangliosides exert no effect on the development of seizures if they are injected in the course of developing kindling and have also no anticonvulsive effect when they are given acutely before a challenge dose of PTZ in PTZ-kindled rats. Interestingly, recently we have found that seizure prevention by diazepam did not result in prevention against learning deficits in PTZ-kindled rats (3). Regarding these results it could be suggested that development of seizures and impairments in learning

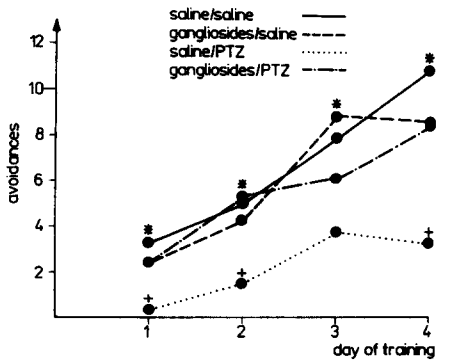

FIG. 3. Effect of gangliosides on the learning performance of kindled and saline-treated rats on 4 daily training sessions in the shuttle-box. Ganglioside injections 1 hour before PTZ or NaCl. *$p<0.05$ saline/saline (n = 13) versus saline/PTZ (n = 13); +$p<0.05$ ganglioside/PTZ (n = 13) versus saline/PTZ (n = 13).

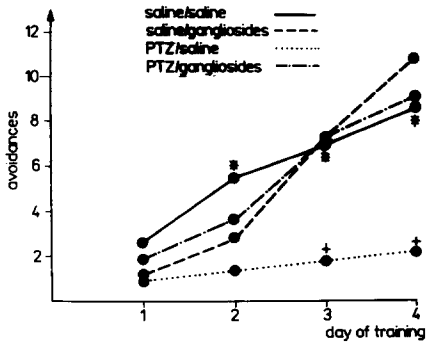

FIG. 4. Effect of gangliosides injected 1 hour before each daily training session on the learning performance of kindled and saline-treated rats on 4 days of training in the shuttle-box. *$p<0.05$ saline/saline (n = 20) versus PTZ/saline (n = 13); +$p<0.05$ PTZ/ganglioside (n = 8) versus PTZ/saline (n = 13).

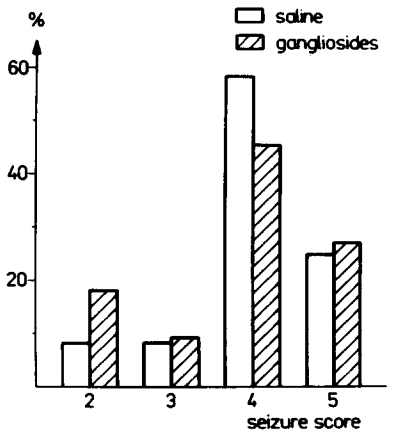

FIG. 5. Effect of 10 mg/kg of gangliosides on the frequency distribution of seizure severity scores induced by 45 mg/kg PTZ in fully kindled rats. Ganglioside group: n = 11; controls: n = 12.

abilities in kindled animals are two different independently developing processes probably founded on one common underlying mechanism.

This leads to the assumption that administration of antiepileptic drugs at doses capable to suppress seizures in epileptic patients is not automatically sufficient to prevent the development of cognitive and mental disturbances. For that reason it seems useful to complete the anticonvulsant therapy with supporting drugs. Regarding the results presented above gangliosides may be such drugs. They are being used therapeutically to treat a variety of nervous system disorders and were able to protect the chemical and morphological changes associated with neural tissue injury (1, 7, 10, 14). Although at present no clear-cut morphological features underlying kindling have been identified the results given above demonstrate a protection against related damages by gangliosides. The effectiveness of the gangliosides using two different administration regimens suggest more than one mechanism of action. The effect with injections in the course of kindling may reflect a prevention of secondary disturbances induced by repeated convulsions and related metabolic changes. It is well accepted that convulsions are followed by hypoxic conditions connected with formation of peroxides resulting in membrane disturbances. Based on the wide range of pharmacological effects of exogenous gangliosides in animal studies, it is hypothesized that gangliosides can protect against changes in plasma membrane structure. Thus gangliosides could act by reducing Ca^{++}-flux and therefore the activation of phospholipases (16), by protection of membrane enzymes like Na,K-ATPase (13) or by reducing ionic imbalance and edema (8,25).

Surprisingly, the gangliosides exert the learning improving effect acutely administered before acquisition sessions, too. In this case mechanisms of protection or enhanced regeneration would not be effective. Perhaps an influence on processes of synaptic transmission (17, 18, 26) may explain this improving effect of the gangliosides.

Although at the moment, the molecular mechanism by which exogenous gangliosides exert their improving effects remains obscure, they could offer a useful possibility to support and ameliorate the basic antiepileptic therapy in the clinic.

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