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The Role of Striatal Glutamatergic System in Haloperidol-Induced Dopamine Receptor Supersensitivity and Effects of Monosialoganglioside GM1

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SCHROEDER, H., U. SCHROEDER and B. A. SABEL. *The role of striatal glutamatergic system in haloperidol-induced dopamine receptor supersensitivity and effects of monosialoganglioside GM1.* PHARMACOL BIOCHEM BEHAV **58**(4) 903–907, 1997.—The mechanism underlying the action of ganglioside GM1 on the increase of haloperidol-induced dopamine receptor supersensitivity was studied using the method of chemically stimulated (^{3}H)-D-aspartate release in rat striatal slices. After a 3-week chronic haloperidol treatment the transmitter release was reduced by about 30%, with a further reduction to 40% when GM1 was applied chronically as well. This suggests that the downregulation of the glutamatergic system by chronic haloperidol treatment is potentiated by gangliosides. The acute effect of gangliosides on the stimulated (^{3}H)-D-aspartate release from striatal slices was tested by adding GM1 to the superfusion medium. When given at a concentration of 10^{-4} M, GM1 did not alter the amino acid release itself. GM1 did, however, reduce the haloperidol-enhanced (^{3}H)-D-aspartate release to control levels and elevated the glutamate-stimulated (^{3}H)-D-aspartate release. Binding experiments indicate that gangliosides do not directly interact with glutamate or dopamine receptors. The data are discussed in view of earlier findings that GM1 potentiates the behavioral supersensitivity following chronic haloperidol treatment without directly altering dopamine receptor supersensitivity. © 1997 Elsevier Science Inc.

(^{3}H)-D-Aspartate release Dopamine Ganglioside Glutamate Binding sites Striatum

GANGLIOSIDES are sialic acid-containing glycosphingolipids present in neuronal cell membranes (22). They have frequently been implicated in various physiologically relevant membrane functions as well as in processes of the restoration and repair of damaged neuronal tissue (14,15).

Recently, we have found that the behavioral expression of the haloperidol (Hal)-induced dopamine receptor supersensitivity can be potentiated by GM1 treatment without affecting striatal D_1- and D_2-binding sites (18). This suggests that gangliosides may act via mechanisms other than dopamine receptors. For example, it is possible that gangliosides may act as a modulator of pre- and postsynaptic processes of the glutamatergic transmission, which in turn, modulate the dopaminergic system by presynaptic interactions, a process that is of interest in many neurodegenerative and psychiatric disorders (2,5). Here, the nigrostriatal dopaminergic and the corticostriatal glutamatergic systems are anatomically and functionally linked (5). It is well known that the K^+-induced glutamate release is inhibited by dopamine (13).

It has already been reported that GM1 affects the c-*fos*-induced opening of the glutamate receptor gated Ca^{2+}-channel, the receptor-mediated phosphoinositide turnover, as well as protein kinases, and that gangliosides are able to prevent glutamate- and related agonist-evoked excitotoxicity in cell cultures (3,11,21).

Therefore, it was of interest to study the reciprocal interaction between dopamine and glutamate in striatum with and without gangliosides in chronic and acute preparations.

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To better understand GM1-stimulated behavioral expression of the Hal-induced dopamine receptor supersensitivity (8) the action of subchronically applied GM1 was examined in the present study. The study focused on those synaptic processes of the glutamatergic transmission that may modulate the activity of the dopaminergic system. In addition, we also studied the acute effects of the gangliosides alone and in combination with glutamate and haloperidol on the stimulated (^{3}H)-D-aspartate release from striatal tissue in vitro. (^{3}H)-D-Aspartate was used as a specific nonmetabolizable marker of glutamate/aspartate release and uptake (6).

METHOD

Animals

The experiments were performed using 8-week-old male Wistar rats of our own breeding stock. Animals were maintained in temperature- and humidity-controlled rooms with a 12-h light–dark cycle. They were housed in plastic cages (five per cage), food and water ad lib. A minimum of six animals were used per group in each experiment.

Haloperidol-Induced Supersensitivity

Rats received daily injections of 1.0 mg/kg IP of Hal for 21 days, whereas controls were given the same number of saline injections. Hal-treated animals of both groups were also treated daily either with GM1 (30 mg/kg, IP, FIDIA Research Labs, Italy) or saline.

Tissue Preparations

On day 24, the animals were decapitated, their brains were rapidly removed, and the striata were dissected out. Crude membrane fractions were prepared by a modified method of Zukin et al. (24). Briefly, to prepare a 3% homogenate (wet weight/volume), tissue was homogenized in 30 mM Tris-HCl buffer, pH 7.4, containing 2.5 mM $CaCl_2$ and stored at −20°C. After thawing, homogenates were centrifuged for 20 min at 50,000 × *g*. The resulting pellet was washed four times with homogenization buffer and centrifuged again.

(^{3}H)-L-Glutamate and (^{3}H)-Spiroperidol Binding Assay

The (^{3}H)-L-glutamate and -spiroperidol binding was measured using a method described by Schröder et al. (16) and Köhler et al. (8). The radiochemical purity of the ligand was checked by TLC and was found to be about 91%. Specific binding was calculated by subtracting nonspecific binding—defined as that seen in the presence of 50 nM (^{3}H)-L-glutamate or 1 nM (^{3}H)-spiroperidol plus 100 μM unlabeled L-glutamate or 2 μM *d*-butaclamol (Serva, Heidelberg, Germany)—from total binding obtained with (^{3}H)-L-glutamate or -spiroperidol alone.

The pellet was suspended with Tris-HCl buffer and 50 μl-aliquots of the crude membrane suspension containing 150–250 g protein were then mixed with (^{3}H)-L-glutamate (specific activity: 1.43 TBq/mmol, NEN-Dupont, USA) or (^{3}H)-spiroperidol (specific activity: 800 GBq/mmol, NEN-Dupont) and were subsequently incubated for 40 min and 30 min at 37°C, respectively. To evaluate the effect of GM1 on both ligand binding assays in vitro the ganglioside was added over a concentration range of 10 nM to 0.1 mM. All assays were performed in at least duplicate.

The reaction was terminated by rapid filtration under reduced pressure through GF/A or GF 10 glass-fiber filters using an Inotech harvester (Berthold, Germany). Filters were washed with buffer and taken for liquid scintillation counting in a toluene containing solvent. The data were determined as fmol bound radioligand per mg protein.

Amino Acid Release

Freshly prepared slices from the whole striatum were incubated with 19.2 nM (^{3}H)-D-aspartate (specific activity 962 GBq/mmol, NEN Dupont) for 10 min under aeration with carbogen (O_2/CO_2—95/5%), transferred into superfusion chambers and rinsed by a calcium-free medium (17). Afterwards, the superfusion was initiated with oxygenated Krebs-Henseleit solution (containing in mM: 118.5 NaCl, 4.7 KCl, 24.9 $NaHCO_3$, 2.5 $CaCl_2$, 2 $MgSO_4$, 1.2 KH_2PO_4 and 10 glucose, pH 7.4) at a flow rate of 0.5 ml/min. After 21 to 25 min (S1) and 31 to 35 min (S2) the medium was replaced by a Krebs-Henseleit solution containing 48 mM KCl. For the acute experiments the S1 phase was measured in combination with GM1, haloperidol (alone or combined with GM1), or glutamate (alone or combined with GM1), each of them in a concentration of 0.1 mM. The S2 of amino acid release from tissue slices of Hal- and GM1-treated rats was determined in the presence of either 0.1 mM Hal or glutamate.

Perfusate was collected in 1-min fractions and assayed for radioactivity in a Beckman 6000 LL counter using a dioxane-containing scintillation cocktail. The purity of labelled D-aspartate in the fractions was checked by HPLC and was found to be about 95%. The tissue slices were homogenized with 0.1 M perchloric acid and the residual radioactivity as well as the protein content were determined. Samples from amino acid release experiments were expressed as dpm per mg protein or as percent of total radioactivity (released and residual radioactivity of slices).

Protein content was estimated in aliquots of tissue homogenates using the technique by Lowry et al. (12) with bovine serum albumine as standard.

Statistics

Data were analyzed with a one-way analysis of variance (ANOVA) and the significance of planned multiple comparisons was calculated using the *t*-test.

RESULTS

(^{3}H)-L-Glutamate and -Spiroperidol Binding

In a first series of experiments we studied the influence of GM1 on the (^{3}H)-L-glutamate and -spiroperidol binding to

TABLE 1

INFLUENCE OF GM1 ON THE SPECIFIC (^{3}H)-L-GLUTAMATE AND -SPIROPERIDOL BINDING TO RAT STRIATAL MEMBRANES

GM1	^{3}H-L Glutamate	^{3}H-Spiroperidol
0 M	2590 ± 319	385 ± 24
10^{-8} M	2029 ± 430	355 ± 30
10^{-7} M	2193 ± 503	386 ± 35
10^{-6} M	2391 ± 286	384 ± 35
10^{-5} M	2237 ± 294	400 ± 32
10^{-4} M	2032 ± 242	398 ± 21

Means of fmol/mg protein ± SEM, $n = 6$.

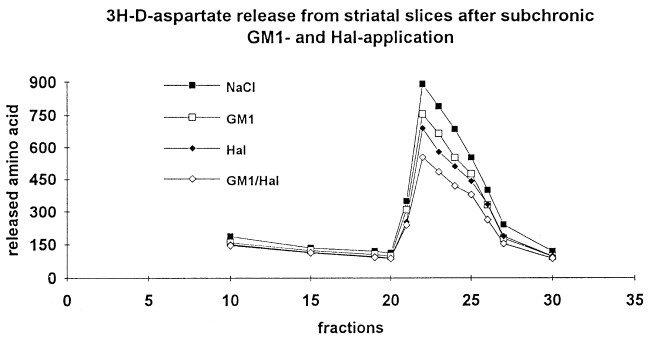

FIG. 1. The collection profile of the K^+-stimulated (^3H)-D-aspartate release from striatal slices of rats treated chronically with Hal, GM1, or Hal/GM1 (stimulation phase 1 in dpm/mg protein, mean ± SEM, $n = 8$).

glutamate and D_2 binding sites in the corpus striatum in vitro. As shown in Table 1, gangliosides did not influence the (^3H)-L-glutamate and -spiroperidol binding to rat striatal membranes.

Amino Acid Release After Chronic Treatment

We studied the K^+-stimulated (^3H)-D-aspartate release from striatal slices of rats that had previously been treated subchronically with Hal alone or in combination with GM1. The values of the S1 of the amino acid release from ganglioside treated rats were comparable to those of the saline controls (Figs. 1 and 2). In contrast, the Hal application was followed by a significant decrease of measured transmitter release ($p < 0.05$, Figs. 1 and 2). When Hal treatment was combined with GM1, this decrease was significantly greater ($p < 0.05$). The addition of 0.1 mM Hal or glutamate to the superfusion medium in the stimulation phase 2 was found to be without any effect on the K^+-stimulated (^3H)-D-aspartate release when striatal slices were taken from Hal-treated rats (Table 2).

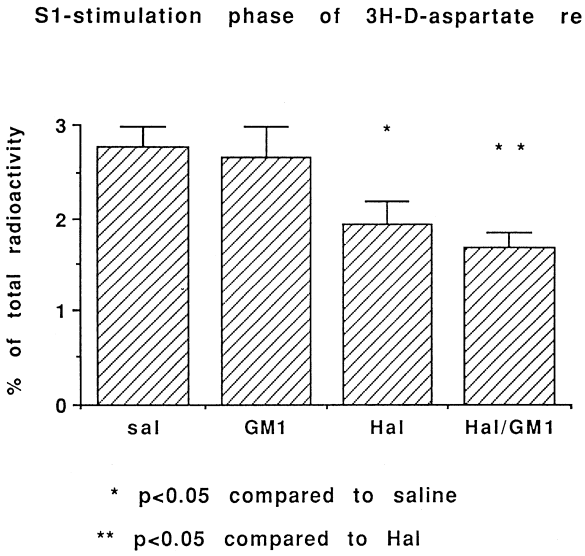

FIG. 2. Comparison of the stimulation phase S1 of the K^+-stimulated (^3H)-D-aspartate release from striatal slices of rats treated subchronically with Hal, GM1, or Hal/GM1 (in dpm/mg protein, mean ± SEM, $n = 7$).

TABLE 2

K^+-STIMULATED (^3H)-D-ASPARTATE RELEASE FROM STRIATAL SLICES OF RATS TREATED SUBCHRONICALLY WITH HAL, GM1, OR HAL/GM1

	Saline	GM1	Hal	GM1/Hal
S2/S1-glu	0.86 ± 0.083	0.92 ± 0.082	0.96 ± 0.087	1.07 ± 0.082
S2/S1-Hal	0.42 ± 0.072	0.39 ± 0.05	0.42 ± 0.055	0.39 ± 0.03

The values represent the ratio of stimulation phase 1 and 2 under the influence of Hal and glutamate, $n = 8$.

Amino Acid Release After Acute Treatment

The K^+-stimulated (^3H)-D-aspartate release from striatal slices remained unchanged also under the acute influence of 0.1 mM GM1 in vitro (Fig. 3). After the addition of 0.1 mM Hal to the K^+-containing superfusion medium amino acid release was enhanced ($p < 0.05$). The combined addition of 0.1 mM Hal and 0.1 mM GM1 was followed by a blockade of the Hal enhanced transmitter release (Fig. 3A).

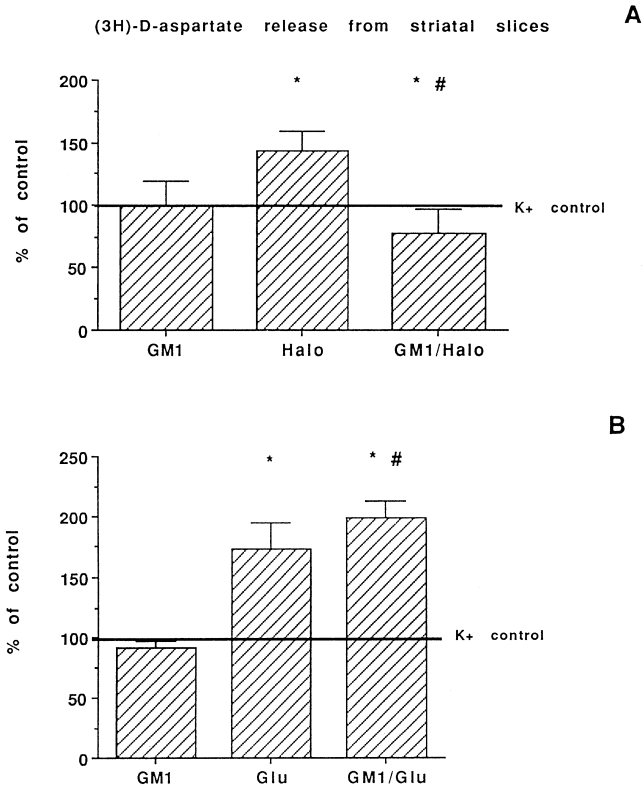

FIG. 3. The influence of 10^{-4} M GM1 on the K^+-stimulated (^3H)-D-aspartate release from striatal slices (stimulation phase 1 in percent of control, mean ± SEM, $n = 6$). (A) Effect of 10^{-4} M GM1 on the HaL (10^{-4} M)-modulated amino acid release, $*p < 0.05$ compared to control (no GM1, no hal), $\#p < 0.05$ compared to the HaL group. (B) Effect of 10^{-4} M GM1 on the glutamate (10^{-4} M)-modulated amino acid release $*p < 0.05$ compared to control (no GM1, no glutamate), $\#p < 0.05$ compared to the glutamate group.

We also studied the effects of glutamate on K^+-stimulated (^{3}H)-D-aspartate release as follows: when 0.1 mM glutamate are added to enhance the K^+-stimulated (^{3}H)-D-aspartate release from slices, GM1 was found to increase the effect of glutamate on stimulating amino acid release from tissue slices ($p < 0.05$, Fig. 3B).

DISCUSSION

We have previously demonstrated that GM1-stimulated behavioral signs of the Hal-induced dopamine receptor supersensitivity cannot be explained by dopaminergic mechanisms (8,18). The present experiments were therefore undertaken to clarify the action of GM1 on the glutamatergic system by assessing striatal slices. We have used both an acute and a chronic assay of the K^+-stimulated (^{3}H)-D-aspartate release that was used as a specific nonmetabolizable marker of glutamate/aspartate release (6). In the acute preparation drugs were added to "naive" striatal slices, while in the chronic preparation the drugs were given to rats prior to preparing brain slices. With this system we also studied specific binding of (^{3}H)-L-glutamate and -spiroperidol to striatal membranes. Because gangliosides did not affect the (^{3}H)-L-glutamate- nor the ^{3}H-spiroperidol binding in the acute experiment, they do not alter the activity of the glutamatergic receptor per se. The chronic treatment with haloperidol leads to an increase in the specific D_2 ligand binding to striatal membranes. As in the previous study (18), chronic GM1 treatment was unable to alter this enhancement of D_2 binding.

Our first main finding is that Hal given chronically to rats inhibits the glutamate release. It is known that Hal, when given chronically, blocks the dopamine receptors on striatal neurons, resulting in an elevation of dopamine receptor density (receptor supersensitivity). However, chronic Hal treatment has also another, perhaps more important, effect in that it inhibits the activity of the striatal glutamatergic system as measured by the glutamate release ex vivo. This downregulation of the glutamatergic system described for humans as a glutamatergic deficiency (19) and determined in rats by microdialysis (7,23) may significantly contribute to the genesis of psychosis and offers a further approach to pharmacotherapy. This inhibition of the glutamatergic system after chronic Hal treatment is significantly potentiated by simultaneous, but not acute, GM1 administration. That this effect cannot be explained by a direct interaction of GM1 on the dopamine or glutamate receptors is indicated by the fact that gangliosides do not alter the affinity of either dopamine- or glutamate-receptors on synaptic membranes. The potentiation of the Hal-effect by GM1 applied simultaneously supports our conclusion that GM1 modulates the activity of the glutamatergic system.

To clarify the effect of GM1 on the glutamatergic system we tested the action of gangliosides on the K^+-stimulated (^{3}H)-D-aspartate release from striatal tissue slices also under acute conditions. Unexpectedly, we found that acute gangliosides reduce Hal-evoked glutamate-release while stimulating glutamate-evoked glutamate-release. As gangliosides do not alter the receptor characteristics at all (see above), this effect is apparently not due to ganglioside action on the receptors themselves. Thus, the inhibited amino acid release after subchronic Hal treament contradicts the acute enhancement of the transmitter release by Hal. This contradictory effect may be explained by the dopamine receptor supersensitivity following subchronic Hal treatment and induced alterations in the glutamatergic transmission.

Furthermore, from the data of the acute experiment it can be postulated that gangliosides might act on second-messenger systems that are involved in the glutamate release. This hypothesis is compatible with the results of others. It has already been shown that GM1 can interact with intracellular processes that are induced by the transmitter receptor interaction: modulation of the activity of phosphokinases, Ca^{2+} fluxes and phosphoinositide turnover (4,11,21).

The opposite action of gangliosides under the condition of glutamate and Hal stimulation in the acute experiment suggests that there are fundamental differences between the dopamine-receptor coupled second-messenger pathways and glutamate receptor-coupled second-messenger pathways, both of which are involved in glutamate release. Further studies are now needed to clarify the precise metabolic events responsible for this interaction.

Because the present chronic experiments show that gangliosides potentiate Hal-induced inhibition of glutamate release, this effect is likely to be the mechanism underlying the behavioral supersensitivity potentiation as shown by Schröder et al. (18). Also, the behavioral potentiation may be due to increased glutamate release as shown during the acute experiment. Such an interaction is conceivable because it is well known that glutamatergic corticostriatal neurons directly interact with striatal dopaminergic fibers and dopaminergic nigrostriatal fibers mediate a presynaptic inhibitory influence on striatal glutamate release (9). On the other hand, using assays with purified striatal synaptosomes Cheramy et al. (1) demonstrated that the synthesis and release of dopamine is regulated by presynaptic glutamate receptors.

According to the known effect that reduced haloperidol at the sigma receptor alters synaptic processes of glutamatergic transmission (3), a possible role of these receptors in the modulation of Hal-induced dopamine receptor supersesitivity should be considered, but further experiments are necessary.

Based on the results reported in this article we propose that a decreased activity of the glutamatergic system as induced by chronic Hal treatment may be the basis for behavioral signs of dopamine receptor supersensitivity, and that this can be potentiated by GM1 treatment.

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