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Short communication

Monosialoganglioside attenuates the excitatory and behavioural sensitization effects of ethanol

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Abstract

The effects of long-term monosialoganglioside G_{M1} treatment on the acute excitatory effects of ethanol and behavioural sensitization to this effect were studied, using locomotion frequency of mice observed in an open field as an experimental parameter. G_{M1} (30 mg/kg, once a day, for 21 days) did not modify mouse behaviour but decreased both the acute excitatory (1.8 g/kg) and the behavioural sensitization effects of ethanol (1.8 g/kg, once a day for 21 days, 30 min after G_{M1} injections). G_{M1} administered acutely 30 min or 24 h before ethanol did not modify the ethanol-induced increase in locomotion frequency. These results agree with previous reports in which ganglioside treatment modified both dopaminergic plasticity and other behavioural and biochemical effects of ethanol.

Keywords: Monosialoganglioside; Ethanol; Sensitization; Locomotor activity

1. Introduction

The excitatory effect of ethanol can be demonstrated when low doses are given. Prolonged administration of low doses of ethanol may produce sensitization, a phenomenon characterized by a progressive increase in locomotor activity after repeated administration of a given dose of psychostimulant drugs (Masur et al., 1986). The stimulating properties of ethanol are a model for the rewarding effects of ethanol in humans and it has been proposed that drug craving is closely related to sensitization, which may increase uncontrollable intake of ethanol. The mechanism of sensitization is not fully clear, but the main focus of studies on this phenomenon has been the mesoaccumbens dopamine projection (Robinson and Berridge, 1993).

Gangliosides, which are natural components of neuronal membranes, seem to play an important role in the events related to dopaminergic plasticity. Exogenous monosialoganglioside G_{M1} co-administration leads to an increase of haloperidol-induced behavioural supersensitivity (Schröder et al., 1994; Vital et al., 1995). Conversely, when G_{M1} is administered after abrupt haloperidol withdrawal, it attenuates significantly the behavioural parameters of dopaminergic supersensitivity (Vital et al., 1995). Numerous experiments have indicated that gangliosides attenuate ethanol-induced neurotoxicity in vitro (Heaton et al., 1994), the effects of ethanol intoxication in vivo (Klemm et al., 1988) and ethanol-induced decreases in locomotion, nose-poke exploration and anxiety (Gilmore et al., 1991).

Based on these data, this study was undertaken to investigate whether G_{M1} would modulate the development of behavioural sensitization to ethanol as well as its excitatory effects.

2. Materials and methods

2.1. Subjects

3-month-old male Swiss mice from our colony (weight 30–35 g) were used. Groups of 20 mice were kept in Plexiglas cages with free access to food and water in a

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room with controlled temperature (22 ± 1°C) and a 12 h light/dark cycle with lights on at 7:00 a.m.

2.2. Drugs

G_{M1} ganglioside (kindly provided by Fidia Research Laboratories) was diluted in distilled water. Ethanol (Merck) was administered as a 12% (v/v) solution also diluted in distilled water. Saline (0.9% NaCl) was used as control solution. All solutions were administered intraperitoneally (i.p.).

2.3. Open-field procedure

The open-field arena was a circular wooden box (40 cm in diameter and 30 cm high) with an open top, and the floor was divided into 18 squares. A circle was marked in the centre of the field. Hand-operated counters were used to score locomotion frequency (number of floor units entered) during 5 min sessions. The studies were always conducted blind and each animal was used only once.

2.4. Blood ethanol measures

The samples were examined by gas chromatography using the headspace technique. Samples were prepared for analysis by diluting 1 part of blood with 1 part of 0.2% aqueous *n*-propanol, as internal standard. The analyses were performed using a 6 foot by 1.8 mm i.d. Porapak QS, 80–100 mesh; column at 200°C with flame ionization detection. Nitrogen served as the carrier gas at a flow rate of 40 ml/min. Detector and injector temperatures were 230°C. Concentrations of ethanol were determined from a previously constructed aqueous calibration curve prepared exactly as were the unknowns.

2.5. Experimental procedures

Four experiments were performed, as follows below.

2.5.1. First experiment

Mice were allocated randomly to four groups of 20–21 animals each, which received either two injections of saline (saline + saline group), G_{M1} and saline (G_{M1} + saline group), saline and ethanol (saline + ethanol group) or G_{M1} and ethanol (G_{M1} + ethanol group), once a day for 21 days. G_{M1} (30 mg/kg) was administered 30 min prior to ethanol (1.8 g/kg). 24 h after the last injection, each group was divided into two subgroups of 10–11 animals each, which were challenged with either saline (-saline) or ethanol (1.8 g/kg) (-ethanol). 5 min later, mice were individually placed in the centre of the open-field arena and their locomotion frequency was quantified.

2.5.2. Second experiment

Mice were allocated randomly to eight groups of 12 animals each, which received acutely two injections of saline, saline + ethanol, 30 mg/kg G_{M1} + saline, 30 mg/kg G_{M1} + ethanol, 60 mg/kg G_{M1} + saline, 60 mg/kg G_{M1} + ethanol, 100 mg/kg G_{M1} + saline or 100 mg/kg G_{M1} + ethanol, respectively. Saline or G_{M1} was administered 30 min prior to saline or ethanol (1.8 g/kg). 5 min after the second injection, mice were observed individually in the open field for locomotion frequency quantification.

2.5.3. Third experiment

40 animals were divided into two equal groups which were treated acutely with either saline or 100 mg/kg G_{M1}. 24 h later, each group was divided into two subgroups which received either saline or 1.8 g/kg ethanol. 5 min later, the animals were observed for locomotion frequency quantification.

2.5.4. Fourth experiment

Mice were allocated randomly to two groups of 20–22 animals each, which received either saline or 30 mg/kg G_{M1} once a day for 21 days. 24 h after the last injection, each group was divided into two subgroups of 10–11 animals each, which received 1.8 g/kg ethanol and were killed 5 or 10 min later in order to take blood samples for determination of blood ethanol levels.

2.6. Statistical analysis

Analysis of variance (ANOVA) followed by Duncan's test was used. A probability of $P < 0.05$ was considered to show significant differences for all comparisons.

3. Results

3.1. First experiment

Fig. 1 shows the effects of long-term saline or G_{M1} administration on the excitatory action of ethanol and on the behavioural sensitization to ethanol. Acutely administered ethanol (saline + saline -ethanol group) had an excitatory effect as compared to the control group (saline + saline -saline group). As expected, due to the low doses administered, ethanol withdrawal did not modify locomotion frequency (the saline + ethanol -saline group did not differ from the saline + saline -saline group). Repeated administration of ethanol induced behavioural sensitization (the locomotion frequency of the saline + ethanol -ethanol group was significantly higher than that of the saline + saline -ethanol group). Long-term treatment with G_{M1} did not produce any effect on locomotion frequency since there was no significant difference between the saline + saline -saline and G_{M1} + saline -saline groups. In addition, G_{M1} treatment did not modify the locomotion frequency of ethanol-withdrawn mice (the G_{M1} + ethanol -saline group did not differ from the saline + ethanol -saline group). However, G_{M1} pretreatment attenuated significantly both the acute excitatory effect of ethanol and the behavioural

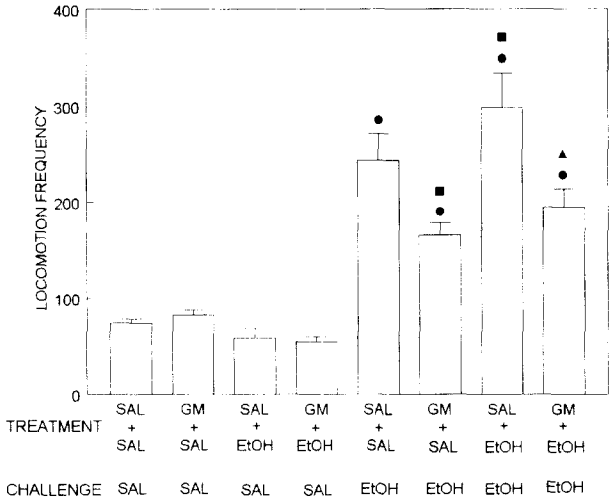

Fig. 1. Effect of long-term G_{M1} (GM) administration on the excitatory action of ethanol (EtOH) and on the behavioural sensitization to ethanol, measured by locomotion of mice in the open field. Results are expressed as means ± S.E.M. ●, $P < 0.05$ compared to SAL + SAL -SAL group; ■, $P < 0.05$ compared to SAL + SAL -EtOH group; ▲, $P < 0.05$ compared to SAL + EtOH -EtOH group (ANOVA followed by Duncan's test).

sensitization induced by repeated administration of ethanol. Indeed, the locomotion frequency of the G_{M1} + saline -ethanol group was significantly lower than that of the saline + saline -ethanol group. Similarly, locomotion frequency of the G_{M1} + ethanol -ethanol group was significantly decreased as compared to that of the saline + ethanol -ethanol group.

3.2. Second experiment

Acute G_{M1} was not effective to reduce the excitatory effect of ethanol, even at higher doses (Fig. 2). In this respect, the three G_{M1} doses used (30, 60 and 100 mg/kg) did not modify locomotion frequency. No significant differences in locomotion frequency were found between the saline + ethanol and G_{M1} (30, 60, 100 mg/kg) + ethanol groups.

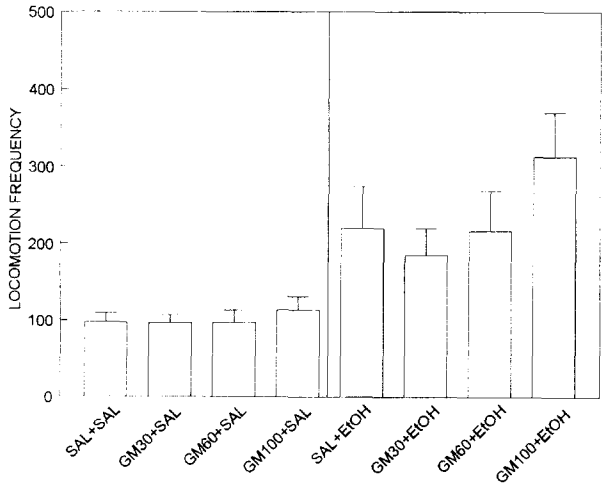

Fig. 2. Effect of the administration of a single G_{M1} (GM) dose (30, 60 and 100 mg/kg) on open-field locomotion of ethanol (EtOH)- or saline (SAL)-treated mice.

3.3. Third experiment

Acute G_{M1} administration was also ineffective to attenuate the excitatory effect of ethanol even when a high dose (100 mg/kg) was administered 24 h before injection of 1.8 g/kg ethanol. Previous administration of 100 mg/kg G_{M1} did not alter the locomotion frequencies of animals treated with either saline or ethanol, as shown by the following values (means ± S.E.M.): 82.3 ± 10.1, 99.9 ± 3.9, 166.4 ± 36.7 and 200.9 ± 26.0 for the saline + saline, G_{M1} 100 + saline, saline + ethanol and G_{M1} 100 + ethanol groups, respectively.

3.4. Fourth experiment

Long-term G_{M1} administration did not modify blood ethanol levels. 5 min after ethanol administration (1.8 g/kg, i.p.), the blood ethanol concentration values (means ± S.E.M.) were 2.55 ± 0.08 and 2.59 ± 0.18 mg/ml for the saline- and G_{M1} long-term-treated mice, respectively. 10 min after ethanol administration, the values were 2.45 ± 0.07 and 2.40 ± 0.1 mg/ml for the saline- and G_{M1} long-term-treated animals, respectively.

4. Discussion

Single intraperitoneal injections of 'depressant' (3.0 g/kg) and 'excitatory' (1.0–2.0 g/kg) doses of ethanol have been shown to produce a marked decrease of whole brain endogenous gangliosides in rodents (Klemm and Foster, 1986). In culture, exogenous gangliosides ameliorate ethanol-induced alterations of membrane gangliosides in fetal and neonatal cortical neurons (Laev et al., 1993). Systemically administered gangliosides cross the blood-brain barrier, regardless of the parenteral route chosen (Bellato et al., 1989), are functionally incorporated into synaptic membranes (Moss et al., 1976) and attenuate the behavioural depressant effects of ethanol (Klemm et al., 1988; Gilmore et al., 1991).

In the light of these results, it is tempting to question whether the G_{M1}-induced attenuation of the excitatory and behavioural sensitization effects of ethanol has anything to do with the ethanol-induced decrease in endogenous sialogangliosides. However, it should be noted that, whereas Klemm and Foster (1986) have shown that acute ethanol treatment greatly reduces the levels of membrane gangliosides, chronic ethanol consumption has little or no effect (see Hungund and Mahadik, 1993). In the present study, long-term G_{M1} administration attenuated both the acute excitatory effect of ethanol and the behavioural sensitiza-

tion produced by *repeated administration* of this substance. Thus, this antagonism between ethanol and exogenous G_{M1} on the membrane content of gangliosides does not seem to be the most likely explanation, at least for the behavioural sensitization results described here.

Both the acute excitatory and the behavioural sensitization effects of ethanol seem to be related to activation of the central dopaminergic system (Robinson and Berridge, 1993). Results of numerous experiments indicate an interaction between gangliosides and dopaminergic function and plasticity (Schröder et al., 1994; Vital et al., 1995). It is therefore possible to speculate that the effects of G_{M1} on ethanol-induced excitatory and sensitization effects are specifically related to modifications of dopaminergic neurotransmission. Interestingly, we have verified that long-term G_{M1} administration decreases striatal dopamine turnover in rats (Vital et al., unpublished results).

There are several other alternative manners in which G_{M1} treatment could inhibit ethanol-induced acute excitatory or behavioural sensitization effects. For instance, gangliosides have been reported to decrease calcium influx into the neuron (Abramson et al., 1972) as well as glutamate receptor-mediated Ca^{2+} functions and excitatory amino acid-induced neurotoxicity (Milani et al., 1991). In this respect, both calcium channel blockers and excitatory amino acid antagonists play important roles in the behavioural effects of ethanol (Littleton et al., 1990; Camarini et al., unpublished results).

From another standpoint, it should be noted that, whatever the mechanism underlying the G_{M1} effects described here, the effects depend on long-term administration. Indeed, acute injections of the glycosphingolipid were ineffective even when higher doses were used at different time intervals before ethanol. Since long-term G_{M1} administration did not modify blood ethanol levels, plastic synaptic modifications seem to be the best explanation for the data reported here. This view is supported by our recent observation that repeated G_{M1} administration also attenuates the behavioural sensitization effects of amphetamine (unpublished data). Since both amphetamine and ethanol behavioural sensitization seem to be closely linked to mesoaccumbens dopamine projection (Robinson and Berridge, 1993), one may hypothesize that G_{M1} long-term administration inhibits a critical cellular mechanism in dopamine mesoaccumbens neurons, such as dopamine D_1/D_2 receptor regulation, Ca^{2+} flux and accompanying intracellular modification, etc. An evaluation of these and other cellular alterations after co-treatment with G_{M1} and ethanol or other compounds has produced behavioural sensitization may be a fruitful avenue for future investigation.

Finally, it should be noted that the excitatory and behavioural sensitization effects of ethanol depend on the species. Masur et al. (1986) showed an increased response of mice to the stimulating effect of ethanol after chronic exposure, whereas rats did not become stimulated by ethanol, either with an acute dose or after chronic treatment. It could be suggested that, although this is highly speculative, differences in endogenous gangliosides levels could account for this species difference.

In conclusion, this study has demonstrated that long-term G_{M1} treatment attenuates specifically the excitatory and behavioural sensitization effects of ethanol. Although the mechanisms related to these findings have yet to be established, the present data extend previous reports about the protective action of gangliosides on the effects of ethanol and suggest a potentially important clinical use of these compounds.

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