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Short communication

Exogenous GM1 ganglioside increases accumbal BDNF levels in rats

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HIGHLIGHTS

- GM1 ganglioside enhances cocaine-conditioned place preference.
- GM1 alone did not induce any conditioning effect.
- GM1 increase the BDNF protein levels in the NAc.

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ABSTRACT

Gangliosides are compounds that are abundant throughout the CNS, participating actively in neuroplasticity. We previously described that exogenous GM1 ganglioside pretreatment enhances the rewarding properties of cocaine, evidenced by a lower number of sessions and/or dosage necessary to induce conditioned place preference (CPP). Since GM1 pretreatment did not modify cocaine's pharmacokinetic parameters, we suspected that the increased rewarding effect found might be mediated by BDNF, a neurotrophic factor closely related to cocaine addiction. This study was performed to investigate the possibility that GM1 may induce changes in BDNF levels in the nucleus accumbens (NAc), a core structure in the brain's reward circuitry, of rats submitted to three conditioning sessions with cocaine (10 mg/kg, i.p.). The results demonstrate that GM1 administration, which showed no rewarding effect by itself in the CPP, induced a significant increase of BDNF protein levels in the NAc, which may account for the increased rewarding effect of cocaine shown in the CPP paradigm.

1. Introduction

Gangliosides, natural components of neuronal membranes, play a significant role in neuroplasticity phenomena. Several lines of evidence have shown that GM1 pretreatment accelerates neuronal adaptive changes induced by several pharmacological treatments [1,2]. Previously, using the conditioned place preference paradigm (CPP), we demonstrated that exogenous GM1, which has no conditioning effect by itself, facilitates cocaine-induced place preference, evidenced by a lower number of sessions and/or a subthreshold dose of cocaine inducing CPP. In order to explore whether GM1 affects cocaine pharmacokinetics, we evaluated brain and plasma esterase activities, plasma bound/free cocaine ratio and brain barrier permeability. Its inhibitory effect on the dopamine transporter was also assessed. However, none of these parameters was modified by GM1 pretreatment [3]. Given that exogenous GM1 has neurotrophic properties, and that brain-derived neurotrophic factor (BDNF) plays an important role in various processes of cocaine addiction, we propose that the impact of GM1 on cocaine rewarding effects might be due to increased BDNF protein levels and/or the influence of BDNF action.

It has been reported that BDNF administration into different structures of the mesolimbic dopamine pathway potentiates psychostimulant effects [4], and that reduced expression of BDNF decreases the rewarding properties of cocaine [5,6]. It has also been proposed that enhanced BDNF in these brain areas may underlie the sensitized response to psychostimulants, cocaine-seeking behavior and conditioned responses to psychostimulants [7]. On the other hand, different lines of evidence indicated that GM1 activates the Trk receptors for neurotrophins in rat brain [8,9]. Furthermore, this activation seems to be induced by the release of neurotrophins, such as neurotrophin-3 (NT-3) and/or BDNF [10,11].

Taking into account that GM1 increases the rewarding properties of cocaine [3], that intra-NAc BDNF infusions increased the cocaine-induced response to the conditioned reinforcer [11], as well as the GM1-BDNF interaction mentioned above, it seems reasonable to propose that GM1 may alter BDNF expression in the reward circuitry. In the present study, to confirm our hypothesis

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that exogenous GM1 may increase levels of BDNF protein in the NAc, we reproduced the same behavioral experimental conditions described previously [3] and then prepared the animals for the BDNF determination.

2. Materials and methods

2.1. Animals

Male Wistar rats from our own colony, weighing 250–330 g, were used in this study, maintained at 22 °C ± 2 under a 12 h light–dark cycle (lights on at 07:00 AM) with free access to food and water. Animals used were maintained and the studies conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (National Research Council, USA, 2010). All efforts were made to minimize both animal suffering and the number of animals used.

2.2. Drugs and treatments

GM1 (dry powder, 98%), obtained from porcine brains, kindly provided by TRB- Pharma, Buenos Aires, Argentina, and cocaine hydrochloride (Verardo Laboratories, Buenos Aires, Argentina) were dissolved in 0.9% saline and administered i.p.

2.3. Conditioning place preference procedure (CPP)

The place conditioning procedure used in the present work was similar to that described by Valdomero et al. [3], and included an unbiased design. The apparatus for CPP consisted of three Plexiglas compartments, the outer two of 32 × 25 × 35 cm (one with black walls and a floor of metal rods; the other with white walls and a floor of polyethylene reticulate). The central compartment, with translucent walls and smooth floor (11 × 22 × 25 cm), was connected to the outer compartments by guillotine doors. Compartments were dimly illuminated during experiments (8 lx).

The CPP procedure consisted of three phases: preconditioning, conditioning and testing. During preconditioning, each animal was placed in the central compartment and allowed to freely explore all three compartments for 15 min. Under our experimental conditions, the animals showed no baseline preference for any of the compartments. Conditioning sessions were conducted twice per day, 5 h apart. Rats were injected with saline and confined to one of the outer compartments for 45 min (pseudo-conditioning session). Five hours later, they were injected with cocaine (10 mg/kg) and confined to the opposite compartment for 45 min. Different groups of animals were pretreated with GM1 (30 mg/kg) or saline 2 h before the corresponding cocaine (10 mg/kg) or saline administration.

With this experimental design, four experimental groups were defined: (I) animals pretreated with saline and paired with saline (SAL-SAL group); (II) animals pretreated with saline and conditioned with cocaine (SAL-COC); (III) animals pretreated with GM1 and paired with saline (GM1-SAL); (IV) animals pretreated with GM1 and conditioned with cocaine (GM1-COC). The conditioning procedure was carried out for a total of three sessions. The treatment compartment was counterbalanced and the presentation order of saline and cocaine alternated. The day following the last conditioning session the CPP test was performed. The animal was placed in the central compartment and, as in the preconditioning test, allowed to explore all three compartments for 15 min. The time spent in each compartment was recorded by two experimenters who were always blinded to the drug treatment and the nutritional conditions of the animals. Place preference was evaluated as time spent in the drug-paired compartment relative to the total time spent in outer compartments [preference score = time in paired/(time in paired + time in non-paired compartment)].

2.4. Analysis of BDNF protein levels

Immediately after the testing session, animals from the different experimental groups were sacrificed by decapitation and the NAc quickly dissected. BDNF protein was extracted from brain tissue by homogenizing the NAc in lysis buffer (137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1% NP40, 10% glycerol, 1 mM PMSF, 10 μg/ml aprotinin, 1 μg/ml leupeptin and 0.5 mM sodium vanadate). Then, samples were centrifuged at 12,000 × *g* for 10 min at 4 °C. The resulting supernatant was removed and diluted 1:5 in DPBS buffer (10 mM NaCl, 2.68 mM KCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 9 μM $CaCl_2{\cdot}2H_2O$, 5 μM $MgCl_2{\cdot}6H_2O$). BDNF levels were assessed using the BDNF Emax ImmunoAssay System kit (Promega, USA) according to manufacturer's recommendations. Briefly, tissue samples were incubated on 96-well flat-bottom plates previously coated with anti-BDNF monoclonal antibody overnight at 4 °C. After blocking, plates were incubated with anti-human BDNF polyclonal antibody for 2 h and anti-IgY antibody conjugated to horseradish peroxidase for 1 h. Then, color reaction with a chromogenic substrate (3,3′,5, 5′-tetramethylbenzidine) was quantified in a plate reader at 450 nm. The standard BDNF curve ranged from 0 to 500 pg/ml.

2.5. Statistical analysis

Differences in behavioral scores were analyzed using two-way analysis of variance (ANOVA) with pretreatment and conditioning drug as independent variables. For BDNF statistical analysis, two-way ANOVA was applied to raw data values of protein levels obtained from the ELISA procedure. Results showing significant overall changes were subjected to the *post hoc* Fisher's LSD test, with values of $P < 0.05$ considered as statistically significant.

3. Results

3.1. Effect of GM1 pretreatment on the rewarding properties of cocaine in the CPP

Fig. 1 shows the place conditioning effect for cocaine (10 mg/kg) in GM1 and saline pre-exposed rats. Two-way ANOVA of the behavioral scores revealed a significant interaction (pretreatment × conditioning drug; $F_{1,32} = 8.94$, $P < 0.01$). In agreement with a previous report from our lab [3], only the GM1-pretreated group showed a significant rewarding effect after 3 conditioning sessions with cocaine 10 mg/kg ($F_{1,32} = 11.63$; $P < 0.005$), with a significant difference found between the GM1-COC and SAL-SAL groups ($P < 0.001$).

3.2. Effect of GM1 pretreatment on accumbal BDNF protein levels

Fig. 2 shows the effect of GM1 pretreatment on BDNF levels in the NAc of rats paired with saline or cocaine. Analysis of the data revealed a significant effect of GM1 pretreatment ($F_{1,32} = 12.51$, $P < 0.001$). *Post hoc* comparison showed a significant increase in BDNF levels in the GM1-SAL and GM1-COC groups compared to their respective control groups (SAL-SAL and SAL-COC, $P < 0.05$).

4. Discussion

The mesolimbic dopaminergic pathway, which projects from the ventral tegmental area of the midbrain to the limbic forebrain including the NAc, is considered one of the most important

Fig. 1. Effect of GM1 pretreatment on cocaine-induced place conditioning. Bars represent mean preference score ± S.E.M. from SAL and GM1 pre-exposed animals conditioned with either SAL or COC (10 mg/kg). Experimental groups consisted of 8-10 rats each. * $P < 0.001$ vs. SAL-SAL group; ** $P < 0.0005$ vs. SAL-COC (LSD Fisher test). SAL, saline; COC, cocaine.

neuroanatomical substrates underlying the rewarding effects of drugs of abuse [12,13]. In a previous work, we demonstrated that GM1 pretreatment increases the capacity of cocaine to induce cocaine place preference, accelerating the process involved in rewarding behavior. This effect was evidenced by the lower dosage of cocaine necessary to induce conditioning compared with rats receiving only cocaine, as well as by the smaller number of sessions necessary to induce place preference [3]. While control rats showed place conditioning after 4 sessions, this effect was observed in GM1-pretreated rats with only 2 or 3 conditioning sessions.

To our knowledge, there is no in vivo evidence reported demonstrating that GM1 enhances brain BDNF protein levels. This study therefore analyzed the influence of exogenous GM1 on BDNF levels in the NAc of rats submitted to 3 conditioning sessions with cocaine. Under our experimental conditions, GM1-pretreated rats, conditioned with cocaine or with saline, have enhanced BDNF levels in the NAc, while cocaine did not modify the protein levels in this

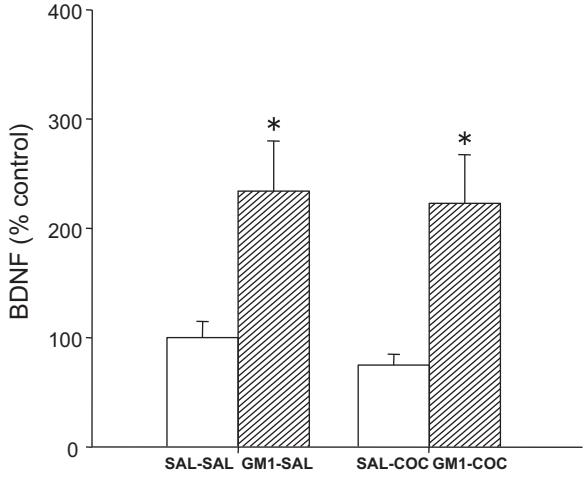

Fig. 2. Exogenous GM1 enhances BDNF protein levels in the NAc. Data are expressed as the percentage of control rats that were pre-exposed and paired with SAL (SAL-SAL group). SAL-COC, animals pretreated with saline and paired with cocaine, GM1–SAL and GM1-COC groups, animals pretreated with GM1 and paired with saline or cocaine, respectively. * $P < 0.05$ vs. their respective SAL-pretreated group. ($n = 10$–12 per group).

brain area. These results are consistent with those of Lim et al. [11] who demonstrated in cell cultures that GM1 induces the release of mature BDNF in hippocampal neurons and both mature BDNF and pro-BDNF in human neuroblastoma cells. Moreover, it is possible that the increase shown in the NAc extends to other brain areas, which may contribute to the neuroprotective/neurotrophic effects of GM1 on dopaminergic neurons previously reported by other authors [14–16], agreeing with Fadda et al. [17] who reported that subthreshold amounts of BDNF were necessary in the presence of GM1 to protect cultured dopaminergic neurons from injury induced by 6-OHDA.

Several studies have reported the involvement of BDNF in various processes closely related to cocaine addiction, such as the rewarding effect and the sensitization phenomenon induced after its administration. Intra-NAc infusions of BDNF enhance cocaine-induced locomotor activity and conditioned reward [4]. In the same way, over-expression of BDNF or TrkB in the NAc increases cocaine CPP [18], and reduced expression of these proteins by genetic manipulation or using an adeno-associated virus (AAV) approach decreases cocaine-induced CPP [5,6].

The CPP paradigm implies associative learning, in which previously neutral cues acquire secondary reinforcing properties when paired with a primary reinforcer [19]. It is known that the NAc is important for learning about environmental stimuli associated with motivationally relevant outcomes. Recently, Brown et al. [20] demonstrated that GABA projections from the VTA regulate accumbal neurons to enhance associative learning. Indeed, there is considerable evidence that BDNF plays a critical role in brain plasticity-related processes such as learning and memory [21–23], and in regulating appetitive and aversive learning [24,25].

In this work, we showed that GM1 pretreatment increases accumbal BDNF protein levels. However, place preference for the side paired with cocaine was only evident in the GM1-COC group and there was no effect in the SAL-COC group. Although GM1 administration alone enhances BDNF levels, it showed no rewarding effect by itself. These observations are consistent with the fact that the CPP paradigm reflects a preference for a context previously paired with a rewarding event. As has been clearly established, cocaine has the ability to induce reward but GM1 by itself does not induce CPP since it has no rewarding properties. The GM1-induced increase in BDNF levels may explain the facilitation of the cocaine rewarding effect demonstrated previously [3], in which only 2 or 3 conditioning sessions with cocaine are sufficient to induce CPP after GM1 pretreatment. Indeed, BDNF may thereby influence the reinforcing properties of cocaine. In view of the well-known involvement of BDNF in drug-associated stimuli, our results suggest that GM1 increases expression of BDNF, which may activate intracellular signaling mechanisms of associative learning that underlie cocaine-induced place preference. However, additional studies must be performed to demonstrate that GM1 facilitates associative learning when an unconditioned stimulus is paired with contextual clues.

5. Conclusion

This study extends previous reports that prove the effects of gangliosides on the modulation of plastic neuronal process. It clearly demonstrates that GM1 increases accumbal BDNF levels. This may lead to altered responsiveness to cocaine, including an increase in cocaine rewarding effects. The cellular and/or molecular mechanism by which GM1 enhances cocaine rewarding properties remains unclear. The elucidation of this phenomenon may contribute to defining therapeutic strategies for the treatment of cocaine addiction.

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