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Short communication

Gamma-butyrolactone-induced dopamine accumulation in prefrontal cortex is affected by tyrosine availability

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ABSTRACT

Gamma-butyrolactone (GBL) elevates striatal and prefrontal cortex dopamine levels; only the striatal dopamine levels are elevated by increased dopamine synthesis. If increased dopamine synthesis is necessary in order for dopamine levels to be affected by tyrosine availability, then GBL-induced prefrontal cortex dopamine levels should be tyrosine insensitive. Rats received either vehicle, tyrosine (50 or 200 mg/kg i.p.) or a tyrosine-depleting mixture prior to GBL 750 mg/kg i.p.. GBL-induced dopamine levels in prefrontal cortex were lowered by tyrosine depletion. GBL-induced striatal dopamine levels were not affected. Hence, increased dopamine synthesis may not be necessary in order for tyrosine availability to affect pharmacologically elevated prefrontal cortex dopamine levels.

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1. Introduction

Dysregulation of tyrosine transport in schizophrenia is associated with cognitive impairments mediated by cortical dopamine systems (Wiesel et al., 2005). Since brain tyrosine availability can affect cortical and subcortical dopamine transmission in man (McTavish et al., 2001; Montgomery et al., 2003) as well as in experimental animals (Milner and Wurtman, 1986; McTavish et al., 1999; Jaskiw et al., 2005), one possibility is that abnormal tyrosine transport contributes to the dopamine dysregulation in schizophrenia (Wiesel et al., 2005). The mechanisms linking tyrosine availability and dopamine transmission remain to be characterized.

One hypothesis states that dopamine synthesis must be increased in order for brain dopamine indices to be sensitive to tyrosine availability (Sved and Fernstrom, 1981). We now describe a test of this hypothesis, exploiting the regional effects of gamma-butyrolactone (GBL). In striatum GBL elevates tissue dopamine levels by elevating dopamine synthesis; in medial prefrontal cortex GBL elevates dopamine levels without affecting dopamine synthesis (Galloway et al., 1986). GBL-induced striatal DA levels are reported to be elevated by increased tyrosine availability and lowered by decreased tyrosine availability (Sved and Fernstrom, 1981). We posited that GBL-induced medial prefrontal cortex dopamine levels would be insensitive to either elevation or lowering of brain tyrosine levels.

2. Methods

Male Sprague–Dawley rats (Zivic–Miller) (175–200 g initial weight) were maintained on a standard 12 hour on/off light cycle with food and water ad libitum in an AALAC accredited facility. Procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Tyrosine methylester hydrochloride (Sigma-Aldrich) was dissolved in distilled water and brought to pH 6.5 with 1 N NaOH. A tyrosine-depleting mixture (NAA(−)) consisting of large and other neutral amino acids (NAAs) (McTavish et al., 1999) was administered in two equal doses one hour apart, at time=−60 min and time=0. Other groups received vehicle at time=−60 min and then vehicle, tyrosine 50 mg/kg or tyrosine 100 mg/kg at time=0. GBL (Sigma-Aldrich) 750 mg/kg i.p. (dissolved in saline) or vehicle was administered at time=30 min. All animals received the same total number of injections. At time=60 min animals were decapitated and brain regions dissected on wet ice (Bongiovanni et al., 2006).

Tissue levels of tyrosine as well as of catechols were measured by high pressure liquid chromatography followed by electrochemical detection (Bongiovanni et al., 2006). Limits of quantitation (expressed per mg protein) were: dopamine 23.5 fmol/mg, DOPAC 46.0 fmol/mg, tyrosine 6.0 pmol/mg. Absolute levels were analyzed by a two-way ANOVA (tyrosine×GBL). If the overall ANOVA drug effect was

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Table 1
Levels (±SEM) of tyrosine (TYR; nmol/mg protein), dopamine (DA; pmol/mg protein), dihydroxyphenylacetic acid (DOPAC; pmol/mg protein) in medial prefrontal cortex (MPFC) or striatum

	VEH/VEH	VEH/GBL	NAA(−)/VEH	NAA(−)/GBL	TYR 50/VEH	TYR 50/GBL	TYR 200/VEH	TYR 200/GBL
	(8)	(10)	(6)	(8)	(8)	(6)	(6)	(8)
MPFC								
TYR	0.95±0.11	0.75±0.05	0.36±0.03[c]	0.29±0.02[c]	1.54±0.11[c]	1.99±0.48[c]	3.55±0.55[c]	3.89±0.23[c]
DA	3.10±0.36	5.78±0.69[a]	2.31±0.35	3.57±0.76[b]	2.95±0.55	4.34±0.63	2.87±0.36	4.39±0.26[a]
DOPAC	5.69±0.76	4.01±0.49	3.88±0.30	3.24±0.58	3.75±0.42[c]	7.01±2.00	7.05±2.04	6.93±2.12
Striatum								
TYR	0.94±0.04	0.71±0.04[c]	0.43±0.03[c]	0.38±0.06[c]	1.60±0.12[c]	1.98±0.97[c]	3.38±0.27[c]	3.30±0.23[c]
DA	395±30	578±36[a]	335±29	532±52[a]	368±33	544±26[a]	369±26	511±26[a]
DOPAC	127±11	102±6	124±19	99.3±7.1	132±7	131±17	133±21	85.1±5.4

Animals initially received vehicle (VEH), tyrosine (TYR) 50 or 200 mg/kg i.p. or a tyrosine-depleting mixture (NAA(−); 1 g/kg i.p.), followed 30 min later by VEH or gamma-butyrolactone (GBL) 750 mg/kg i.p. and were sacrificed after an additional 30 min. Significant differences ($P<0.05$); a — compared to VEH counterpart; b — compared to VEH/GBL; c — compared to VEH/VEH; numbers in parentheses = number of animals/group.

significant, individual groups were compared by post-hoc *t*-tests corrected for multiple comparisons. Pearson correlations were determined between dopamine, DOPAC and tyrosine levels. Data are expressed as mean±S.E.M.

3. Results

For tyrosine levels in medial prefrontal cortex, there were significant effects of tyrosine (F (3,48)=68, $P<0.0001$) but not of GBL ($F(1,48)=0.72$, $P=0.4$) or of GBL×tyrosine interaction ($F(3,48)=1.1$, $P=0.4$). As expected, tyrosine levels were lowered by NAA(−) and elevated by tyrosine 50 mg/kg i.p. or 200 mg/kg i.p. (Table 1).

For dopamine in medial prefrontal cortex, there was a significant effect of GBL ($F(1,48)=3.9$, $P<0.05$), but not of tyrosine ($F(3,48)=0.70$, $P=0.6$) or of GBL×tyrosine interaction ($F(3,48)=0.54$, $P=0.7$). Post-hoc tests showed significant dopamine elevation in the groups "vehicle/GBL" and "tyrosine 200 mg/kg/GBL" ($P<0.05$) and a trend to significant elevation in the group "tyrosine 50 mg/kg/GBL" ($P<0.06$). The "NAA(−)/GBL" group had dopamine levels similar to those of "NAA(−)/vehicle" animals and was the only GBL-treated group with levels significantly lower than those of "vehicle/GBL" animals ($P<0.05$). Thus, GBL significantly elevated medial prefrontal cortex dopamine levels and NAA(−) attenuated the GBL effect (Fig. 1, Table 1). The ANOVA for DOPAC did not show significant effects. However, there was a significant correlation overall between tissue DOPAC and tyrosine ($r=0.8$; $P<0.02$).

For striatal tissue tyrosine levels, there were significant effects of tyrosine ($F(3,49)=182$, $P<0.0001$) but not of GBL ($F(1,49)=0.01$, $P=0.9$) or of the GBL×tyrosine interaction ($F(3,49)=1.9$, $P=0.2$). As

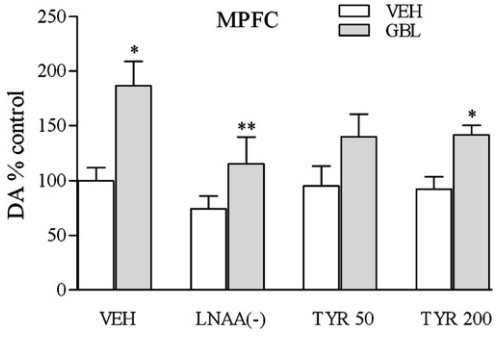

Fig. 1. Dopamine (DA) levels in medial prefrontal cortex (MPFC) were measured in groups that received an initial treatment with vehicle (VEH), a tyrosine-depleting amino acid mixture (NAA(−)), tyrosine 50 mg/kg (TYR 50) or tyrosine 200 mg/kg (TYR 200) followed 30 min later by VEH or gamma-butyrolactone (750 mg/kg). All treatments were administered i.p.. Tissue was harvested 30 min after GBL. DA levels are expressed as % of controls (VEH/VEH). Significantly different ($P<.05$), * — compared to VEH counterpart, ** — compared to VEH/GBL.

expected, tyrosine levels were significantly lowered by NAA(−) and elevated by tyrosine 50 mg/kg i.p. and 200 mg/kg i.p. (Table 1). GBL itself lowered striatal tyrosine levels in vehicle pretreated rats, but not in other groups. For dopamine in striatum, there was a significant effect of GBL ($F(1,48)=31$, $P<0.0001$) but not of tyrosine ($F(3,48)=1.3$, $P=0.3$) or of GBL×tyrosine ($F(3,48)=0.88$, $P=0.5$). GBL significantly elevated striatal dopamine levels in every group and this elevation was not affected by any of the other treatments (Table 1). DOPAC in striatum showed a significant effect of GBL ($F(1,48)=9.43$, $P<0.004$) but not of tyrosine ($F(3,48)=1.46$, $P=0.2$) or of the GBL×tyrosine interaction ($F(3,48)=1.30$, $P=0.3$). DOPAC levels did not differ between the groups. There was no significant correlation between tyrosine and either DOPAC or dopamine.

4. Discussion

Contrary to our hypothesis, the GBL-induced dopamine accumulation in medial prefrontal cortex was sensitive to brain tyrosine levels (Fig. 1, Table 1). This suggests that the relationship between tyrosine availability and dopamine levels is more complex than initially anticipated (Sved and Fernstrom, 1981).

GBL inhibits impulse propagation in dopamine neurons (Roth et al., 1973) and lowers terminal region extracellular fluid dopamine levels (Bongiovanni et al., 2006). In striatum, the resulting lower occupancy of synthesis-modulating terminal dopamine autoreceptors leads to increased phosphorylation of tyrosine hydroxylase (Lew et al., 1999) and increased dopamine synthesis (Walters and Roth, 1976; Galloway et al., 1986). With exocytotic release inhibited, the newly synthesized dopamine accumulates (Walters and Roth, 1976; Bannon et al., 1981). Thus in striatum the GBL-induced increase in tissue dopamine is driven by increased dopamine synthesis (Bannon et al., 1981; Galloway et al., 1986). In contrast, mesocortical dopamine terminals possess only release modulating autoreceptors and a small, rapidly turning over but highly releasable dopamine pool (Cooper et al., 2002). GBL elevates tissue medial prefrontal cortex dopamine levels by inhibiting dopamine release but without affecting dopamine synthesis (Bannon et al., 1981; Galloway et al., 1986). We exploited these brain regional differences by measuring GBL-induced dopamine accumulation while varying brain tyrosine availability.

Brain tyrosine levels were lowered (40–45% control) by systemic administration of NAA(−), a mixture of neutral amino acids that compete with tyrosine for transport across the blood-brain barrier (McTavish et al., 1999). Tyrosine levels were elevated by tyrosine administration (50 mg/kg — 165% control; 200 mg/kg — 370% control). Changes in tyrosine levels alone did not affect tissue dopamine levels (Table 1). GBL significantly elevated dopamine levels in medial prefrontal cortex and striatum as expected (Bannon et al., 1981). In