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SHORT COMMUNICATIONS

Effect of D-lysergic acid diethylamide on striatal choline acetyltransferase activity in the rat

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Neuroleptic-induced catalepsy is modulated primarily via mutually antagonistic dopaminergic–cholinergic interaction in the striatum [1, 2]. The intensity of the cataleptic response elicited by acute haloperidol administration correlates significantly with enhanced striatal choline acetyltransferase (acetyl-CoA:choline-*O*-acetyltransferase; ChAT; EC 2.3.1.6) activity [3]. More recently, some investigators [4] have drawn attention to the possible importance of central cholinergic mechanisms in schizophrenia and related affective disorders and, hence, it is of interest to investigate whether the hallucinogen-induced behavioral syndromes are associated with changes in cholinergic activity. Previous studies of the neurochemical substrates of D-lysergic acid diethylamide (LSD)-induced catalepsy suggest a modulatory role of cholinergic function in this behavioral paradigm.* In the present communication, we attempt to correlate the possible changes in striatal ChAT activity with dose-dependent cataleptic responses after acute LSD treatment.

Male Sprague–Dawley rats (320–360 g) obtained from the Canadian Breeding Farm, Quebec, were used. The animals were housed in groups of three in plastic cages and maintained on a 12-hr (6:00 a.m. to 6:00 p.m.) light schedule at 21°. Water and Purina rat chow were provided *ad lib.* Prior to the start of the experiments, they were allowed to acclimatize for at least 3 days in the animal quarter.

LSD and 2-bromo-LSD were supplied by the Health Protection Branch, Health and Welfare, Canada, Ottawa, and prepared in a 0.9% saline solution for subcutaneous injection in a volume of 1 ml kg^{-1} of body weight. Tritiated acetyl-CoA (sp. act. 1.97 Ci/m-mole) was purchased from New England Nuclear, Boston, MA, U.S.A., physostigmine from the Sigma Chemical Co., St. Louis, MO, U.S.A., and other reagent-grade chemicals from various commercial sources.

The intensity of catalepsy was evaluated as described previously [5]. Essentially, the procedure entailed gently placing both front paws of the rats in extended positions on a horizontal metal bar at 10 cm above a wooden platform in a sound-attenuated room. The length of time for which the animal remained in this abnormal posture was recorded, the cut-off time limit being set at 120 sec. At specified time intervals after LSD injection, the maximal cataleptic response among three trials was reported. All drug treatments and behavioral testing were carried out between 9:00 a.m. and 7:00 p.m. Animals were decapitated at 2.5 or 20 hr after the acute LSD challenge.

The method of radiochemical assay for ChAT was slightly modified from that of Fonnum [6]. Animals were killed in a 5° room, and subsequent steps were carried out on ice unless specified otherwise. The striata from the decapitated rats were homogenized in 20 × vol. of 10 mM EDTA (pH 7.4 at 25°) containing 0.1% (v/v) Triton X-100 and 5% (w/v) bovine serum albumin. The tissue was homogenized by hand through 15 up–down strokes in an all-glass homogenizing tube (13 × 100 mm, Corning 7725). The reaction mixture comprised 1 M sodium phosphate buffer (pH 7.4 at 25°) containing 3 M NaCl, 150 mM EDTA, 1.5 mM acetyl-CoA (with [acetyl-^{3}H]acetyl-CoA, 10^6 cpm/ml), 80 mM choline bromide and 2 mM physostigmine. The tissue homogenate (15 μl) was added to 15 μl of the reaction mixture in a 12 × 75 mm tube and, after mixing, was incubated at 37° for 7 min in a constant shaking water bath. The reaction was terminated by adding 1 ml of ice-cold 10 mM sodium phosphate buffer (pH 7.4 at 25°). The content of the test tube was vortexed and transferred to a 7-ml glass scintillation vial. One ml of 0.005% (w/v) sodium tetraphenylboron in acetonitrile and 4 ml of scintillation fluid (PPO–POPOP–toluene, 0.5:0.02:100, w/w/v)† were added to the vials and the vials were inverted gently ten times by hand to mix the contents. The radioactivity in each vial was determined in a Beckman

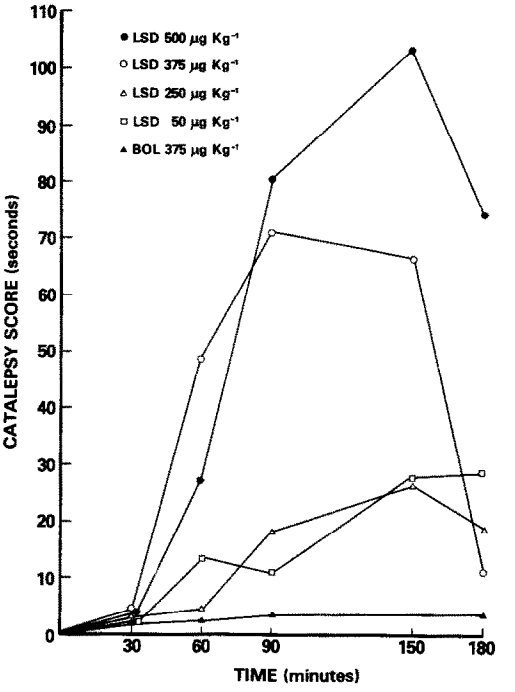

Fig. 1. Time course of the dose-dependent cataleptic response evoked by LSD. Various doses of LSD (50–500 $\mu g\ kg^{-1}$) and a 375 $\mu g\ kg^{-1}$ dose of BOL were administered subcutaneously and the intensity of catalepsy (in sec) was recorded at 30, 60, 90, 150 and 180 min respectively, following LSD administration, as described previously. Each point represents the mean cataleptic score of six animals.

* S. Chiu, and R. K. Mishra, manuscript submitted for publication.

† PPO = 2,5-dephenyloxazole; and POPOP = 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene.

Table 1. Effect of acute LSD administration on ChAT activity in the rat neostriatum*

Treatment	ChAT activity (nmoles ACh formed/mg protein/hr) 2.5 hr post-LSD†	20 hr post-LSD
Saline control (1 ml kg^{-1})	80.6 ± 3.3 (6)	83.4 ± 2.5 (7)
LSD (50 $\mu g\ kg^{-1}$)	117.6 ± 8.0‡ (6)	89.7 ± 3.3 (6)
LSD (250 $\mu g\ kg^{-1}$)	89.6 ± 4.9 (6)	90.3 ± 2.8 (6)
LSD (375 $\mu g\ kg^{-1}$)	79.5 ± 2.1 (6)	83.8 ± 1.6 (6)
LSD (500 $\mu g\ kg^{-1}$)	ND§	78.9 ± 2.4 (6)

* Results are expressed as means ± S.E.M. The number of animals examined is shown in parentheses.

† The 2.5 hr interval was chosen because the maximal cataleptic responses occurred then.

‡ Significantly different from control ($P < 0.05$).

§ Not determined.

LS-230 liquid scintillation counter and was corrected for quench effects by employing the external standard channel ratio. ChAT activity is expressed as nmoles acetylcholine (ACh) formed/mg of protein/hr.

The data were evaluated statistically by analysis of variance followed by the Duncan multiple-range test [7]. The difference between the experimental and control values was considered to be statistically significant at $P < 0.05$.

Within 10 min after LSD administration (50–500 $\mu g\ kg^{-1}$, s.c.) the rats exhibited hyperexcitability and augmented responses to auditory and tactile stimuli in a dose dependent manner. After the initial phase of hyperexcitability, the animals appeared to be relatively immobile and displayed signs of catalepsy at 60 min after LSD administration. As shown in Fig. 1, 50 $\mu g\ kg^{-1}$ failed to produce any appreciable degree of catalepsy, whereas higher doses of LSD (375 μg and 500 $\mu g\ kg^{-1}$) induced significant cataleptic reactions, the maximum intensity of which was attained approximately 2.5 h after LSD injection. On the other hand, the non-hallucinogenic congener of LSD, 2-bromo-LSD (BOL), at a dose of 375 $\mu g\ kg^{-1}$, failed to elicit any biphasic behavioural effects.

The effects of various doses of LSD on ChAT activity in the neostriatum at 2.5 hr and 20 hr are summarized in Table 1. It appears that the changes in striatal ChAT activity do not correlate with the intensity of the LSD-induced cataleptic responses, since the cataleptogenic dose of LSD (375 $\mu g\ kg^{-1}$) failed to alter the ChAT activity. In contrast, 50 $\mu g\ kg^{-1}$ of LSD significantly increased the striatal ChAT activity, as compared with the saline control ($P < 0.05$; 46 per cent increase). Furthermore, in the 250 $\mu g\ kg^{-1}$ group, the striatal ChAT activity was elevated slightly (11 per cent increase over the saline control), although not to a statistically significant degree. At 20 hr after LSD administration, however, the ChAT activities in the LSD-treated groups did not differ significantly from that in the saline control, and the behavioral effects previously elicited by LSD disappeared. Hence, we could not demonstrate any dose-dependent and time-related relationship regarding the effects of LSD on striatal ChAT activity. Accordingly, the intensity of the dose-dependent LSD-induced cataleptic responses appears to be divorced from the changes in ChAT activity.

Although Lloyd *et al.* [3] claimed that enhancement in striatal choline acetyltransferase activity correlated significantly with the development of an acute haloperidol-induced cataleptic response, our results with the prototypal hallucinogen LSD fail to reveal such a relationship. This appears to be surprising in view of the demonstrated high affinity of LSD for the dopamine (DA) and haloperidol binding sites in the calf striatal membranes [8] and its inhibitory effect on DA-sensitive adenylate cyclase activity [9]. It should be emphasized, however, that LSD also exhibits concomitant dopamine agonist activity, as reflected in its stimulatory action on basal activity of adenylate cyclase [10, 11], which is considered to be an integral component of the DA receptor [12]. Electrophysiological evidence also lends support to the DA agonist action of LSD [13]. It is conceivable that, at higher doses of LSD, its intrinsic agonist activity tends to predominate over its antagonistic effect and hence this may partially explain the failure to observe any dose-dependent effect of LSD on striatal ChAT activity. Indeed, Menon *et al.* [14] observed that higher doses of LSD progressively stimulated locomotor activity in the reserpinized animal preparation, and haloperidol reversed the LSD-induced excitatory behavioral effect. A similar trend was discerned by Trulson *et al.* [15] in the nigrostriatal lesioned rotational model.

If LSD does exhibit augmented dopamine agonist activity at higher doses, we would not anticipate any dose–response cataleptic effect. Since we do show such relationship, the cataleptic responses produced by LSD may, after all, reflect a direct interaction with central cholinergic mechanisms. Previous studies in our laboratory indicated that, while muscarinic agonists attenuated LSD-induced catalepsy, muscarinic antagonists potentiated the cataleptic phenomenon.* Analysis of the interaction of cholinergic agonists and antagonists with neuroleptics like haloperidol and chlorpromazine revealed a diametrically opposite trend: enhanced cholinergic activity potentiated neuroleptic-induced catalepsy, whereas decreased cholinergic activity antagonized it [2]. This further raises the possibility that LSD may act on some extra-striatal site in this behavioral paradigm.

Although dopaminergic–cholinergic interaction in the striatum constitutes the primary determinant in the pathogenesis of extra-pyramidal motor deficits associated with neuroleptic therapy [16], changes in ChAT activity may not be a sensitive biochemical index of cholinergic function. Clozapine does not elicit any extra-pyramidal side-effects in humans [17, 18], yet it does not inhibit the striatal ChAT activity in rats [19]. Recent investigations have emphasized the importance of high affinity choline uptake in modulating the *in vivo* synthesis of ACh [20]. Perhaps it would be more meaningful to utilize changes in ACh turnover as a reliable biochemical parameter to evaluate cholinergic functional activity, since the release of ACh may also influence the turnover of ACh.

In the final analysis, the hallucinogenic property of LSD may be related to its interaction with the cholinergic receptor. Although it has been commonly accepted that LSD-induced hallucinosis arises from the interaction with serotonergic and/or dopaminergic receptors [15, 21], this proposition has

* S. Chiu, and R. K. Mishra, manuscript submitted for publication.

recently been challenged by Pieri *et al.* [22], who indicated that the non-hallucinogenic ergot derivative, lisuride, produced similar neurochemical changes. The hallucinogen phencyclidine, which is structurally related to LSD in possessing the phenylethylamine moiety and elicits cataleptic responses, has been demonstrated to interact with the muscarinic receptors [23] and to exhibit cross-tolerance with cholinomimetic agents [24]. The extent to which the hypothesized interaction with the muscarinic receptor contributes to the psychotomimetic activity of LSD remains to be determined. Accordingly, the effects of LSD and its analogues on ACh turnover and choline uptake, as well as its interaction with the cholinergic receptors *in vitro* and *in vivo*, merit further investigation.

In conclusion, we have demonstrated that, whereas a low dose of LSD (50 $\mu g\ kg^{-1}$, s.c.) significantly increased striatal ChAT activity at 2.5 hr, higher doses exerted no appreciable effects. The intensity of LSD-induced cataleptic responses appears to be dissociated from changes occurring in the ChAT activity.

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Neuropharmacology Laboratory, Departments of Psychiatry and Neurosciences, McMaster University Medical Centre, Hamilton, Ontario L86 4J9, Canada

YU-WAH WONG
SIMON CHIU
RAM K. MISHRA

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Effect of selenite on drug-induced methemoglobinemia in rats

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We reported previously that selenite stimulates the reduction of methemoglobin (metHb) in nitrite-treated erythrocytes *in vitro* [1]. We also demonstrated that the reduction of metHb by thiol compounds, such as reduced glutathione (GSH), is increased by the presence of some selenocompounds, suggesting that this catalytic effect of selenocompounds may participate in the stimulation of metHb reduction observed in nitrite-treated erythrocytes [2]. In fact, selenite had no stimulatory effect in erythrocytes depleted of GSH by *N*-ethylmaleimide treatment [3]. This paper reports that selenite suppresses drug-induced methemoglobinemia (metHbemia) in rats, and its mode of action is discussed.

Male Sprague–Dawley rats (7-weeks-old) were used. Blood samples were obtained by cardiac puncture in heparinized syringes. The techniques used for *in vitro* experiments with washed rat erythrocytes were as described previously [1]; experimental details are described in the legend of Fig. 1. MetHb was determined by the procedure of Evelyn and Malloy [4] and hemoglobin (Hb) by the cyanmethemoglobin technique. Glutathione peroxidase (GSH-Px) activity was measured by a coupled reaction with glutathione reductase using cumene hydroperoxide as substrate, according to the method of Prohaska and Ganther [5]. NADH-metHb reductase was measured by the method of Hegesh *et al.* [6], selenium (Se) by the method of Watkinson [7], and protein by the method of Lowry *et al.* [8]. All chemicals used were of reagent grade.

Figure 1 shows the suppressive effect of selenite on metHbemia induced by aniline or phenylhydrazine in rats. The metHbemia produced by aniline (100 mg/kg, i.p.) increased to a peak level within 30–60 min after aniline injection and then gradually decreased. Selenite, when injected simultane-