# LSD and dopamine-sensitive adenylate-cyclase in various rat brain areas

P. F. SPANO, K. KUMAKURA*, G. C. TONON, S. GOVONI AND M. TRABUCCHI

*Institute of Pharmacology and Pharmacognosy, University of Milan and (M.T.) Institute of Pharmacology, University of Milan, Milan 20129 (Italy)*

(Accepted April 14th, 1975)

The mechanism or mechanisms by which LSD induces its hallucinogenic effects are not yet understood[1,2,6,7]. Biochemical, physiological and behavioral data suggest that LSD interacts at the level of monoaminergic synapses in brain[9,17].

Recently Pieri and Pieri[14] have observed that LSD, as apomorphine, induces circling behavior in rats which had been unilaterally injected in the medial forebrain bundle with 5,6-dihydroxytryptamine or 6-hydroxydopamine. The circling behavior induced by LSD or apomorphine in the lesioned rats was blocked by haloperidol, indicating that LSD, like apomorphine, may stimulate directly dopamine (DA) receptors in the striatum.

On the other hand, Uzunov and Weiss[19] have described that D-LSD produces an increase of the concentration of cyclic AMP either when incubated *in vitro* with slices of rat brain stem or in rat brain stem and cerebrum after *in vivo* administration. Moreover, these authors showed that the neuroleptic trifluoperazine both *in vitro* and *in vivo* inhibits the accumulation of cyclic AMP elicited by D-LSD. However, Uzunov and Weiss have left open the question of how D-LSD increased the concentration of cyclic AMP in brain since none of the psychotomimetic drugs they studied altered the activity of either adenylate cyclase or phosphodiesterase in cerebrum, brain stem, cerebellum or pineal gland homogenates.

More recently DA-sensitive adenylate-cyclases have been identified in homogenates of the rat caudate nucleus[10], tuberculum olfactorium[5], nucleus accumbens[8] and limbic cortex[4]. Haloperidol and other antipsychotic neuroleptics antagonize the activation by DA of this enzyme.

It was therefore our purpose to investigate whether D-LSD directly and selectively stimulates DA-sensitive adenylate-cyclase in striatum and in other areas of the limbic system which appears to be more strictly linked to perception, feeling and emotional behavior.

Male Charles River rats, weighing 120–150 g, were used in our experiments.

* Visiting scientist from the Dept. of Biochemistry, Sophia University, Tokyo, Japan.

Adenylate-cyclase activity was measured in the striatum, nucleus accumbens and tuberculum olfactorium, limbic cortex, cerebellum and retina following the method of Kebabian *et al.*[10] with minor modifications. After 3 min of incubation the reaction was stopped by boiling the samples for 3 min. The cyclic AMP was purified and assayed using the method of cyclic AMP dependent protein kinase described by Kuo and Greengard[11]. In each experiment some samples were incubated in the presence of $5 \times 10^{-6}$ *M* DA, normally producing an increase of 100% of the basal activity, to test the accuracy of the experimental conditions.

D-LSD tartrate (United Pharmaceutical Works, Prague) was solubilized in distilled water. For *in vivo* experiments control rats were treated also with distilled water. The 2-bromo-D-lysergic acid diethylamide bitartrate (BOL) was a gift from Zambon Laboratories (Italy). The proteins were measured according to Lowry *et al.*[12].

The *in vitro* addition of D-LSD ($2 \times 10^{-5}$ *M*) to homogenates of rat brain areas, where DA-sensitive adenylate-cyclases have been described, significantly stimulates the formation of cyclic AMP over the basal activity (Table I). In striatum, nucleus accumbens, tuberculum olfactorium and limbic cortex the increase of the formation of cyclic AMP after D-LSD ranges from 30 to 40%. However, in the cerebellum where neither dopaminergic terminals nor a DA-sensitive adenylate-cyclase have been described, the addition of D-LSD has no effect. This last result corroborates the data by Uzunov and Weiss[19] and points out the specific effect of D-LSD on adenylate-cyclases sensitive to dopamine.

The specificity of the effect of D-LSD is further substantiated by the results shown in Table II, where it can be seen that BOL, which is devoid of hallucinogenic properties, has no effect on DA-sensitive adenylate-cyclase in rat striatum.

Moreover, from the results reported in Table II it appears that D-LSD, in addition to being a specific DA agonist, reduces the maximal stimulating effect by DA. In fact the activation of the enzyme by DA is inhibited if D-LSD itself is added to the incubation medium. Furthermore the stimulation of cyclic AMP formation by DA ($10^{-5}$ *M*) in striatal homogenates is apparently inhibited by about 40% when

TABLE I

ADENYLATE-CYCLASE ACTIVITY IN DIFFERENT RAT BRAIN AREAS AFTER *in vitro* INCUBATION WITH LSD $2 \times 10^{-5}$ *M*

Values expressed as pmoles cyclic AMP formed/min/mg protein. Values for cyclic AMP formed are means (± S.E.M.) of at least 12 determinations.

| *Brain areas* | *Controls* | *LSD 2 × 10⁻⁵ M* | *Stimulation (%)* |
|---|---|---|---|
| Striatum | 278 ± 16 | 390 ± 12* | 40 |
| Nucleus accumbens | 240 ± 14 | 337 ± 17* | 39 |
| Tuberculum olfactorium | 141 ± 9 | 182 ± 7* | 29 |
| Limbic cortex | 167 ± 7 | 222 ± 14* | 33 |
| Cerebellum | 50 ± 8 | 48 ± 7 | — |

* $P < 0.001$.

TABLE II

EFFECT OF LSD, BOL, DOPAMINE ALONE OR IN COMBINATION WITH LSD ON ADENYLATE CYCLASE ACTIVITY IN RAT STRIATAL HOMOGENATES

Values for cyclic AMP formed are means (± S.E.M.) of at least 12 determinations.

| *Treatment* | *Cyclic AMP formed (pmoles/min/mg protein)* |
|---|---|
| Controls | 240 ± 13 |
| LSD $2 \times 10^{-5}$ *M* | 336 ± 18* |
| BOL $2 \times 10^{-5}$ *M* | 227 ± 20 |
| DA $10^{-5}$ *M* | 468 ± 21* |
| LSD $2 \times 10^{-5}$ *M* + DA $10^{-5}$ *M* | 390 ± 19*,** |

* $P < 0.001$ when compared to control values.
** $P < 0.001$ when compared to DA values.

D-LSD is administered *in vivo* (250 μg/kg i.p. 15 min before killing — data not shown). These data indicate a common site of action for DA and D-LSD on the DA-sensitive adenylate-cyclase system.

Our results give strong support to the hypothesis of a direct effect of D-LSD on the DA-sensitive adenylate-cyclases which have been associated with the dopaminergic receptors in brain. This effect is apparent not only in the striatum but in those areas of the brain where dopaminergic terminals have been described, *i.e.*, nucleus accumbens, tuberculum olfactorium and limbic cortex.

Most important in our view is the fact that these areas are part of the limbic brain which may play an important role in the psychotomimetic action of D-LSD. Moreover, although drug-induced psychoses and schizophrenia may well be distinct entities[15,16,18], the specific antidopaminergic action of neuroleptics, which can abort LSD syndrome[13], acquires a new meaning after the demonstration of a direct action of D-LSD on thed opaminergic receptors of the limbic system. On the other hand, we have observed in a separate series of experiments (unpublished observations) that D-LSD ($10^{-6}$ *M*) activates a DA-sensitive adenylate-cyclase in the retina[3] of different animal species.

However, the effect of D-LSD on DA receptors in the striatum and limbic system is not the only important central effect of D-LSD. Due to the molecular structure of this compound (an indole ring) the possibility that D-LSD exerts certain of its effects through interaction with central serotonin pathways has received considerable attention[1,7]. It might well be that D-LSD possesses different actions at different monoamine receptors in the brain thus having an extraordinary powerful and rapid pharmacological effect. The possible role of dopaminergic receptors in the hallucinogenic action of D-LSD is presently under further investigation in our laboratory.

When this manuscript was in preparation we learned of a series of experiments on the striatal adenylate-cyclase, performed by Von Hungen *et al.*[20], fully supporting our conclusions.

1 ANDÉN, N.-E., CORRODI, H., FUXE, K., AND HÖKFELT, T., Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide, *Brit. J. Pharmacol.*, 34 (1968) 1–7.
2 BROEKKAMP, C. L. E., PIJNENBURG, A. J. J., AND VAN ROSSUM, J. M., Dopaminergic transmission in relation to mechanisms underlying stereotyped behavior. In E. USDIN AND S. H. SNYDER (Eds.), *Frontiers in Catecholamine Research*, Pergamon Press, New York, 1973, pp. 675–676.
3 BROWN, J. H., AND MAKMAN, M. H., Stimulation by dopamine of adenylate cyclase in retinal homogenates and of adenamine-3:5-cyclic monophosphate formation in intact retina, *Proc. nat. Acad. Sci. (Wash.)*, 69 (1972) 539–543.
4 CARENZI, A., SPANO, P. F., AND TRABUCCHI, M., Dopamine-sensitive adenylate-cyclase in the limbic cortex of rat brain, *Pharmacol. Res. Commun.*, (1975) in press.
5 CLEMENT-CORMIER, Y. C., KEBABIAN, J. W., PETZOLD, G. L., AND GREENGARD, P., Dopamine-sensitive adenylate cyclase in mammalian brain: a possible site of action of antipsychotic drugs, *Proc. nat. Acad. Sci. (Wash.)*, 71 (1974) 1113–1117.
6 FOG, R., Stereotyped and non-stereotyped behaviour in rats induced by various stimulant drugs, *Psychopharmacologia (Berl.)*, 14 (1969) 299–304.
7 HAIGLER, H. J., AND AGHAJANIAN, G. K., Lysergic acid diethylamide and serotonin: a comparison of effects on serotonergic neurons and neurons receiving a serotonergic input, *J. Pharmacol. exp. Ther.*, 188 (1974) 688–699.
8 HORN, A. S., CUELLO, A. C., AND MILLER, R. J., Dopamine in the mesolimbic system of the rat brain: endogenous levels and uptake mechanism and stimulation of adenylate cyclase activity, *J. Neurochem.*, 22 (1974) 265–270.
9 JOHNSON, A. M., VIGOURET, J. M., AND LOEW, D. M., Central dopaminergic actions of ergotoxine alkaloids and some derivatives, *Experientia (Basel)*, 29 (1973) 763.
10 KEBABIAN, J. W., PETZOLD, G. L., AND GREENGARD, P., Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain and its similarity to the 'Dopamine receptor', *Proc. nat. Acad. Sci. (Wash.)*, 69 (1972) 2145–2149.
11 KUO, J., AND GREENGARD, P., An assay method for cyclic AMP and cyclic GMP based upon their abilities to activate cyclic AMP-dependent and cyclic GMP-dependent protein kinases. In P. GREENGARD, R. PAOLETTI AND G. A. ROBISON (Eds.), *Advances in Cyclic Nucleotide Research, Vol. 2, New Assay Methods for Cyclic Nucleotides*, Raven Press, New York, 1972, pp. 41–50.
12 LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J., Protein measurement with the Folin phenol reagent, *J. biol. Chem.*, 193 (1951) 265–275.
13 MURPHREE, H. B., Quantitative studies in humans on the antagonism of lysergic acid diethylamide of chlorpromazine and phenoxybenzamine, *Clin. Pharmacol. Ther.*, 3 (1962) 314–320.
14 PIERI, L., AND PIERI, M., Lysergic acid diethylamide(LSD)-induced rotation in rats with two types of unilateral chemical neurotoxic lesions of the median forebrain bundle (MFB), *J. Pharmacol. (Paris)*, 5, Suppl. 2 (1974) 77.
15 SNYDER, S. H., BANERJEE, S. P., YAMAMURA, H. I., AND GREENBERG, D., Drugs, neurotransmitters and schizophrenia, *Science*, 184 (1974) 1243–1253.
16 STEVENS, J. R., An anatomy of schizophrenia?, *Arch. gen. Psychiat.*, 29 (1973) 177–189.
17 STONE, T. W., Further evidence for a dopamine receptor stimulating action of an ergot alkaloid, *Brain Research*, 72 (1974) 177–180.
18 TORREY, E. F., AND PETERSON, M. R., Schizophrenia and the limbic system, *Lancet*, ii (1974) 942–946.
19 UZUNOV, P., AND WEISS, B., Psychopharmacological agents and the cyclic AMP system of rat brain. In P. GREENGARD, R. PAOLETTI AND G. A. ROBISON (Eds.), *Advances in Cyclic Nucleotide Research, Vol. 1, Physiology and Pharmacology of Cyclic AMP*, Raven Press, New York, 1972, pp. 435–453.
20 VON HUNGEN, K., ROBERTS, S., AND HILL, D. F., LSD as an agonist and antagonist at central dopamine receptors, *Nature (Lond.)*, 252 (1974) 588–589.