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The acceptability of oral contraceptives for women has led many people to investigate the possibility of producing similar products for use by men[1]. The major mechanism of action of synthetic steroid hormones in oral contraceptives is inhibition of ovulation, due to suppression of the mid-cycle surge of gonadotrophins released by the pituitary[2]. Oral contraceptives inhibit spermatogenesis by a similar mechanism, but so far there has been concomitant loss of libido and potency[3]. Clinical results[4-6], however, have shown that this can be avoided if supplementary androgens are given in combination with an anti-gonadotrophic steroid such as danazol[4], norgestrienone[5], or ethylnorgestrienone[6]. But clinical testing of such combinations on a large scale cannot begin without extensive toxicological testing with animals[7]. We have therefore reconsidered existing oral hormone products which have been approved for sale in many countries. One group of these products[8] contains combinations of an androgen (usually methyltestosterone: 17α-methyl-17β-hydroxy-4-androsten-3-one) with an oestrogen (usually ethynyloestradiol: 17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol). They are sold for treatment of, for example, osteoporosis in men and the symptoms of 'male menopause'.

Initial studies were conducted on two men with osteoporosis who had been receiving products of this type for several months. Both proved to be aspermic, but reported no undesirable side effects, apart from a transitory nausea during the first few days. Concentrations of bilirubin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase and albumin in these men were within normal limits. With this background, we decided to test an oral androgen–oestrogen product as a contraceptive in healthy male volunteers.

The concentration of testosterone in the plasma of healthy men averages 4–12 ng ml^{-1}, with a daily production of 5–10 mg[9]. Methyltestosterone has a longer biological halflife than testosterone, but a 10 mg tablet is necessary to maintain concentrations of 4–15 ng ml^{-1} in the plasma for up to 8 h (ref. 10). We therefore used tablets containing 10 mg methyltestosterone plus 20 μg ethynyloestradiol. This combination was taken twice daily at 0800h and 1800h with meals. A recent study[11] suggested that this dose of oestrogen is sufficient to suppress pituitary secretion of follicle-stimulating hormone (FSH) in normal adult men. To evaluate possible side effects we compared the active hormone preparation with a placebo in the same patients. As supplies of matching placebo tablets were unavailable, the active tablets were crushed and repacked in gelatin capsules. Identical placebo capsules were packed with the same quantity of lactose. Five healthy male subjects (23–38 yr old) received daily placebo capsules for 3 weeks before taking the hormone product, but were unaware of this. The wives of the subjects all used combined-type oral contraceptives and continued to do so until advised to the contrary.

Blood and semen were collected at the start of the study and after 3, 6, 12, 18 and 24 weeks. Routine biochemical analysis of blood plasma indicated no hepatotoxicity. All semen specimens were normal at the beginning and end of the period when placebo was given, but sperm numbers and motility had decreased significantly by week 12 (that is day 63 of hormone treatment). At this time, four of the subjects were aspermic and remained so for the duration of treatment. The other subject produced an aspermic semen specimen during week 18. Semen specimens were collected at approximately monthly intervals after treatment was stopped. There was no increase in sperm in any of the men for about 15 weeks after the cessation of hormone treatment, but all then began to show a gradual restoration. Normal sperm numbers and motility were found after 35–40 weeks without treatment.

We conclude that the androgen oestrogen combination inhibited spermatogenesis, but that the effect was reversible when treatment stopped. Two subjects reported decreased libido during the initial 8 weeks (which included the placebo period), but normal libido for the remainder of the study. Another man reported increased libido during the second half of the treatment period, while he and another subject experienced a reduction in libido after treatment was stopped.

Three subjects reported occasional mild nausea, sometimes while they were taking the placebo. No nausea was reported after week 18. There were no changes in skin, hair, breasts or micturation. When the wives of the volunteers stopped taking their oral contraceptives between weeks 18 and 34, no pregnancies occurred, as would have been expected with aspermic men.

Thus we believe that hormone preparations already being marketed for other purposes could be used as oral contraceptives for men. We recommend that these preparations be investigated further to establish the most suitable doses and administration regimen. It seems possible that similar effectiveness could be obtained with discontinuous treatment.

MICHAEL BRIGGS
MAXINE BRIGGS

Biochemistry Department,
Alfred Hospital,
Melbourne 3181, Australia

Received September 23, 1974.

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TIERI

LSD as an agonist of dopamine receptors in the striatum

THE mechanism of the hallucinogenic action of (+)-lysergic acid diethylamide (LSD) is still obscure. Its molecular structure (containing both an indole and a phenylethylamine moiety) suggests the possibility of an interaction with brain monoamines. The earliest hypothesis postulated an antagonistic action of LSD at 5-hydroxytryptamine (5-HT) receptors in the brain[1,2]. More recent data rather tend to support the view that the hallucinogen mimics at least some of the effects of endogenous cerebral 5-HT[3-5]. Inhibition of the release of 5-HT is another suggested mechanism of action[6]. An interaction of LSD with dopaminergic transmission has so far not been demonstrated, although the marked attenuation of LSD-induced symptoms by chlorpromazine and other antipsychotic drugs[7-9] may suggest this. Using a modification of the rotational model proposed by Ungerstedt[10] we found that LSD acted as a potent agonist at dopamine receptors in the striatum.

A unilateral lesion of the nigro-striatal dopamine system of rats lowers the dopamine content in the striatum on that side. The resulting imbalance between the dopaminergic activities in the right and left striatum leads to postural and locomotor asymmetry both spontaneously and, even more markedly, in response to certain agents. Thus, drugs which enhance the release of dopamine from nerve terminals in the striatum of the intact side, for example, amphetamine, induce a rotation towards the side with the

lesion (ipsilateral circling) possibly by removing an inhibitory influence of the striatum upon the motor activity of the side of the body contralateral to the lesion. Conversely, agents like apomorphine, which stimulate dopamine receptors directly, induce rotation towards the intact side (contralateral circling), probably due to exaggerated responsiveness of the denervated striatum to these agents[10].

Unilateral lesions of the nigro-striatal dopamine system were produced in male SPF rats of the stock Füllinsdorf albino by the injection into the right medial forebrain bundle (MFB) of either 3.5 μg 6-hydroxydopamine (6-OH-dopamine) in 4 μl Ringer's solution containing 0.02% ascorbic acid, or 10 μg 5,6-dihydroxytryptamine (5,6-HT) in 10 μl of same. Using these two neurotoxic agents we obtained two groups of animals similarly depleted of telediencephalic dopamine on the side of the injection but differing in the levels of the other monoamine transmitters: 6-OH-dopamine lowered dopamine and noradrenaline levels (L.P., M.P., and W.H., in preparation), but did not alter the 5-HT content; the injection of 5,6-HT depleted dopamine and 5-HT but did not affect noradrenaline[11,12]. For the measurement of drug-induced behaviour, rats were gently placed in a plexiglas box (35×20×15 cm) immediately following the intraperitoneal (i.p.) injection of the compounds. Handling alone induces in most lesioned animals a 'paradoxical' contralateral rotation[10], however, which never lasts more than 3 min. The circlings during the first 5 min were therefore not recorded. The intensity of the rotational behaviour was expressed as the total number of full turns counted during 2 min at intervals of 5 min. Beginning on the 10th postoperative day, all rats were tested for their rotational responses to (+)-methamphetamine and apomorphine before LSD was given. A drug-free interval of one week was allowed between tests. As expected, the responses to (+)-methamphetamine (1-5 mg kg^{-1} i.p.) and apomorphine (0.05-3 mg kg^{-1} i.p.) were ipsilateral and contralateral circling, respectively. Animals treated with 6-OH-dopamine and with 5,6-HT did not differ in their responses. Ten sham-operated rats (local injection of solvent into the MFB) did not rotate in response to apomorphine and amphetamine.

LSD (tartrate) (100 μg kg^{-1} i.p., but not 50 μg kg^{-1} i.p.) induced contralateral turning, which began after 5 min and reached a peak after 15 min (Fig. 1). Doses higher than 100 μg kg^{-1} did not increase further the number of turns per minute but the peak of rotational behaviour was prolonged in a dose dependent way. The time-course of rotation after LSD (100 μg kg) paralleled the temporal changes of the levels of LSD in the brain[13]. Repeated injections of LSD (200 μg kg^{-1} i.p.) on 5 consecutive days, a schedule reported to induce tolerance to the hallucinogen in operant behaviour studies[14], did not reduce the rotational responses. There was no significant difference between rats lesioned with 6-OH-dopamine and those lesioned with 5,6-HT in the intensity and duration of contralateral circling in response to LSD (200 μg kg^{-1}) (Fig. 2). In both groups of animals the injection of haloperidol (1 mg kg^{-1} i.p.) 10 min after the administration of LSD (200 μg kg^{-1}) significantly reduced the circling behaviour (Fig. 2). Pretreatment 4 h before LSD, with α-methyl-L-tyrosine (α-MT) (200 mg kg^{-1} i.p.), an inhibitor of the synthesis of catecholamines, did not alter the rotational response in spite of producing marked sedation. (+)-2-Bromo-lysergic acid diethylamide bitartrate (BOL-148), up to 20 mg kg^{-1} i.p., was unable to induce circling in either group of lesioned animals. LSD in the doses used had no detectable effect in sham-operated rats.

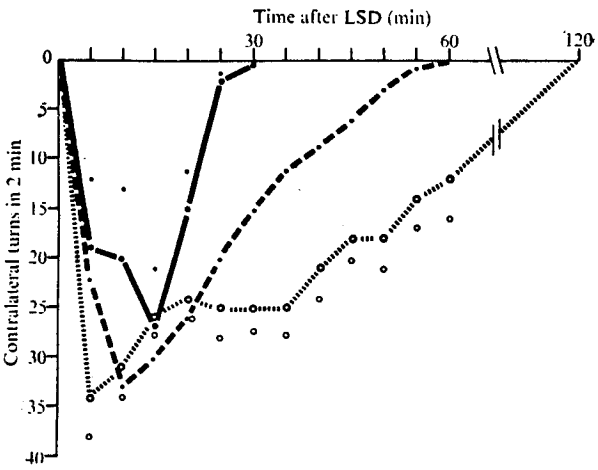

Fig. 1 Contralateral rotation (towards the unoperated side) induced by three doses of LSD injected i.p. in rats with lesions of the right medial forebrain bundle produced by the local injection of 5,6-dihydroxytryptamine. The intensity of the rotational behaviour is indicated by the number of turns in 2 min (means of ten animals per dose) s.e.m. Indicated by small points or circles. LSD doses: ———, 100 μg kg^{-1}; — — — 200 μg kg^{-1}; ····· 1,500 μg kg^{-1}.

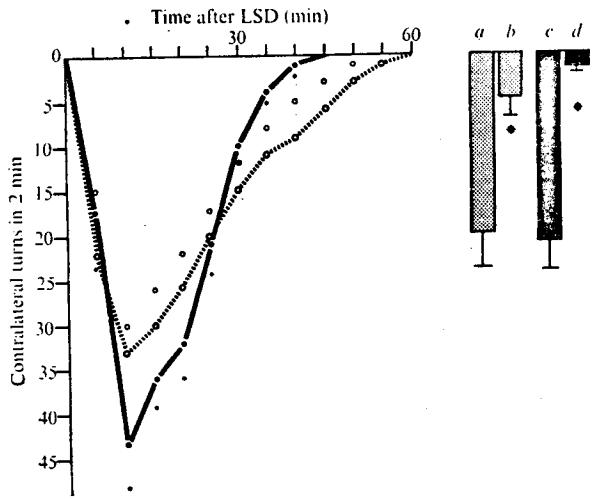

Fig. 2 Comparison of the rotational response elicited by LSD (200 μg kg^{-1} i.p.) in rats with lesions of the right medial forebrain bundle produced by 6-hydroxydopamine ($n=10$) and by 5,6-dihydroxytryptamine ($n=10$). The right-hand side shows the effect of haloperidol (1 mg kg^{-1}) injected 10 min after LSD. *a* and *c*, Turning activity in the two groups of rats, 25 min after LSD alone; *b* and *d*, turning activity under the effect of haloperidol. Means ± s.e.m. ($n=10$). The difference between LSD alone and LSD+haloperidol is statistically significant ($P<0.01$, Student's *t* test, two-tailed). Solid line and bars, 6-hydroxydopamine; dotted line and stippled bars 5,6-hydroxytryptamine.

Thus, in rats with the nigro-striatal dopamine system lesioned unilaterally by injection into the MFB of two different neurotoxic agents (6-OH-dopamine and 5,6-HT), LSD induced circling towards the intact side exactly as does apomorphine. It seems reasonable to conclude that LSD is a potent agonist at central dopamine receptors. Differential depletion of noradrenaline and 5-HT in addition to the reduction of dopamine and the inhibition of catecholamine synthesis by α-MT had no influence on the effect of LSD; therefore, the mechanism involved in the rotational response to LSD seems to be purely dopaminergic and postsynaptic. Haloperidol, a dopamine receptor antagonist, reduced the effect of LSD as well as that of apomorphine (and of L-dopa). Further support for a dopamine receptor agonistic action of LSD is provided firstly by its slowing down of the turnover of dopamine in the rat brain (M. Da Prada, personal communication), secondly by its ability, though weaker than that of DA, to activate *in vitro* the adenyl cyclase in a homogenate of rat striatum (W. P. Burkard, personal communication), and finally by the increase of

cyclic adenosine 3',5'-monophosphate in rat cerebrum after its i.p. administration[15]. BOL-148, an analogue of LSD believed to have very weak, if any, hallucinogenic properties[61], did not induce circling behaviour. In preliminary experiments, methysergide, another non-hallucinogenic lysergic acid derivative, and N,N-dimethyltryptamine, an idolealkylamine hallucinogen, were also inactive as dopamine receptor agonists. On the other hand, such an action has recently been described for some ergot alkaloids and derivates (ergocornine, 2-bromo-α-ergocryptine, ergometrine)[17,18].

The activation of striatal dopamine receptors is obviously not the only important central effect of LSD, since its overall behavioural effects differ considerably from those of agents believed to be fairly pure agonists of dopamine receptors. The possible importance of the dopaminergic component of the action of LSD for its hallucinogenic effect has yet to be demonstrated. It will be interesting to see whether LSD also activates dopamine receptors in brain regions other than the striatum, for example, in the limbic subsortical and cortical[18,19] structures, in the hypothalamus, in the medullary chemical trigger zone, and in the retina[22,23].

We thank Dr A. Kaiser of the Chemical Research Department for 6-OH-dopamine and 5,6-HT.

LORENZO PIERI
MARGHERITA PIERI
WILLY HAEFELY

Department of Experimental Medicine,
F. Hoffmann-La Roche & Co. Ltd.,
Grenzacherstrasse 124,
Basle, Switzerland, CH4002

Received August 15; revised September 20, 1974.

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LSD as an agonist and antagonist at central dopamine receptors

THE mechanisms involved in the psychotomimetic actions of D-lysergic acid diethylamide (D-LSD) and other hallucinogenic agents have not been defined. Neurophysiological and behavioural studies indicate that D-LSD may interact with serotonin and catecholamine receptors in the central nervous system. Thus, this drug seems to stimulate certain central serotonergic pathways[1,2], while inhibiting the activation of other pathways by serotonin[3,4].

D-LSD has also been reported to block the action of noradrenaline on central neurones[5] and to produce stereotypy in rats[6], a condition which is associated with dopaminergic hyperactivity[7]. Moreover, several recent reports have indicated that a number of ergot derivatives are capable of exerting dopamine-like actions on central neurones in the rat[8–10]. Recent investigations from this laboratory suggest that adenyl cyclase systems may be involved in the central actions of D-LSD (ref. 11 and K.v H., S.R., and D.F., unpublished). Thus, D-LSD is capable of stimulating adenyl cyclase activity, as well as antagonising the stimulation of this enzyme by serotonin, in cell-free preparations from the rat colliculus. Here we report evidence that D-LSD may act both as an agonist and an antagonist at dopamine receptors in the brain.

The methods employed for the preparation of brain subcellular fractions and measurement of adenyl cyclase activity have been described in detail elsewhere[12,13]. Brains were obtained from young, adult male rats of an inbred Sprague–Dawley strain. These rats were approximately 6 weeks old and weighed about 225 g. Particulate fractions were prepared from cerebral cortex or corpus striatum.

In relatively low concentration (10 μM), D-LSD, completely blocked the stimulation of cerebral adenyl cyclase activity by a combination of 10 μM noradrenaline and 10 μM dopamine (Table 1). At these concentrations, the catecholamines produced additive effects on enzyme activity[12]. Another serotonin antagonist, 2-bromo-D-lysergic acid diethylamide (BOL) also inhibited this response to the catecholamines. Comparable results were obtained when these serotonin antagonists at 100 μM concentrations were tested against equimolar amounts of either noradrenaline or dopamine. D-LSD also inhibited

Table 1 Influence of lysergic acid derivatives on activation of adenyl cyclase by catecholamines in particulate preparations from cerebral cortex of adult rats

Lysergic acid derivative	Cyclic AMP formed: Basal (nmol mg^{-1} protein h^{-1})	Cyclic AMP formed: Increase with catecholamines (nmol mg^{-1} protein h^{-1})	(%)
None	8.46±0.18	6.39±0.22	76
D-LSD, 10 μM	9.46±0.26	0.31±0.29*	3
L-LSD, 80 μM	8.02±0.15	5.63±0.49	70
BOL, 10 μM	7.60±0.28	0.91±0.41*	12

The particulate preparations were crude mitochondrial fractions obtained by centrifuging the 1,000*g* supernatant fluids from cerebral homogenates at 10,000*g* for 20 min. Adenyl cyclase activity was determined from the conversion of ^{14}C-ATP to cyclic AMP by a procedure[12] which was based on the method of Krishna *et al.*[14]. The incubation medium (0.1 ml) contained the following components in the final concentrations indicated: 3.6 mM (0.5 μCi) 8-^{14}C-ATP, 5.0 mM $MgCl_2$, 1.0 mM cyclic AMP, 40 mM Tris-HCl (*p*H 7.3 at 37° C), 10 mM phosphoenolpyruvate, 4 μg pyruvate kinase, 15 mM $(NH_4)_2SO_4$ (added with pyruvate kinase), 20 mM caffeine, 0.1 mg bovine serum albumin, 0.2 mM EGTA, 5 μg phosphatidylserine and 50–100 μg brain protein. Protein was measured by the method of Lowry *et al.*[15]. L-Noradrenaline (10 μM) and dopamine (10 μM) were added together as the hydrochlorides, D-LSD and L-LSD as the free amines and BOL as the bitartrate. Incubation was conducted in air for 5 min. Values for cyclic AMP formed are averages ±s.e. for triplicate samples in a representative experiment. Where indicated, basal values shown were obtained in the presence of the lysergic acid derivative.

*$P < 0.001$ for the difference between this value and the value obtained with only the catecholamines added.