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DIRECT INTERACTION OF LSD WITH CENTRAL "BETA"-ADRENERGIC RECEPTORS

Annette Dolphin[1], Alain Enjalbert[1], Jean-Paul Tassin[2], Marguerite Lucas[1] and Joël Bockaert[1].

Laboratoire de Physiologie Cellulaire[1] and Groupe NB (INSERM U 114)[2], Collège de France, 11 place Marcelin Berthelot, 75231 PARIS CEDEX 05, France.

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SUMMARY

(+)-lysergic acid diethylamide (LSD) has been found to interact with central β-adrenergic receptors, both in the cerebral cortex of the rat and in cultured C 6 glioma cells. LSD inhibited the binding of ^{3}H-dihydroalprenolol (^{3}H-DHA) with an apparent inhibition constant of 10^{-7}M in the cerebral cortex and 10^{-6}M in C 6 glioma cells. The displacement of ^{3}H-DHA binding by LSD was found to be competitive in the cortex, with a dissociation constant of 1.9×10^{-7}M, compared to 1.4×10^{-8}M for (-)alprenolol and 5.6×10^{-8}M for (-)isoproterenol. BOL, an analogue of LSD without hallucinogenic properties, showed the same affinity as LSD for the cortical β-adrenergic receptor. However, several dopamine and serotonin agonists and antagonists were without effect at 10^{-6}M. The stimulation of adenylate cyclase by isoproterenol was inhibited by LSD with an apparent inhibition constant of 1.6×10^{-7}M in homogenates of cerebral cortex and 5×10^{-6}M in the C 6 glioma cell system. The results suggest that central β-adrenergic receptors represent one of the several sites of action of LSD.

In an attempt to elucidate the complex psychomimetic action of (+)-lysergic acid diethylamide (LSD), its direct interaction with various central monoaminergic receptors has been widely investigated. Its action on cerebral serotonin receptors was the first to be reported. LSD was found to compete with ^{3}H-serotonin binding (1) and to be a partial agonist of serotonin-sensitive adenylate cyclase (2). More recently LSD has also been found to interact with cerebral dopamine (DA) receptors, both from binding (3) and adenylate cyclase studies (4, 5). However, these two effects of LSD are not sufficient to explain all its behavioural actions, and there is indirect evidence to suggest that LSD is able to interfere with noradrenergic neurotransmission (6 - 8). Additionally, Greenberg et al (9) have reported an inhibition of α-adrenergic agonist and antagonist binding by LSD.

There are also several reports which indicate that β-adrenergic antagonists have an action on central serotonin receptors (10-12), and this has been suggested as a mechanism for the antipsychotic properties of propranolol (12).

In the present study we have investigated the interaction of LSD and other serotonin agonists and antagonists with central β-adrenergic receptors, in order to clarify the extent of cross-reactivity between drugs acting on serotonin and β-adrenergic receptors. The properties of central β-adrenergic

receptors have been investigated in rat cerebral cortex and in cultured C 6 glioma cells, both by receptor binding, using ^{3}H-dihydroalprenolol (^{3}H-DHA) a specific ligand of β-adrenergic receptors (13, 14, 15) and by measuring (-)-isoproterenol-sensitive adenylate cyclase.

METHODS

In the rat cerebral cortex homogenates, the binding of ^{3}H-DHA was performed according to the method of Bylund and Snyder (14). The cerebral cortex was dissected as described by Glowinski and Iversen (16). The activity of (-)-isoproterenol-sensitive adenylate cyclase was measured in homogenates of the molecular layer of the cerebral cortex by the method of Bockaert et al (17). In homogenates of C 6 glioma cells ^{3}H-DHA binding and (-)-isoproterenol-sensitive adenylate cyclase were measured as described previously (18).

Drugs and Biochemicals.

(-)-isoproterenol was obtained from Sigma, and dopamine (DA) from Calbiochem. The following coumpounds were kindly donated : (-)-alprenolol and phentolamine (Ciba-Geigy), 2-bromo-(+)-lysergic acid diethylamide (BOL), LSD and methysergide (Sandoz), methergoline (Farmitalia, Milan), methiothepin maleate (Hoffmann la Roche), 5-methoxy-N-N-dimethyltryptamine (5M-DMT) and bufotenine (Regis), fluphenazine (Squibb), sulpiride (Delagrange), mescaline and serotonin creatine sulfate (Merck). (-)-^{3}H-dihydroalprenolol (32-36 Ci/mmole), ^{3}H cyclic AMP, ammonium salt (25 Ci/mmole) and α-^{32}P-ATP sodium (10-20 Ci/mmole) were purchased from New England Nuclear.

RESULTS

As previously described (17), the β-adrenergic sensitive adenylate cyclase in homogenates of rat cerebral cortex had an apparent affinity for (-)-isoproterenol of 1.6 x 10-^{7}M. Increasing concentrations of LSD inhibited this response, with an apparent inhibition constant (K_I app.) of 10-^{7}M (Fig 1A).

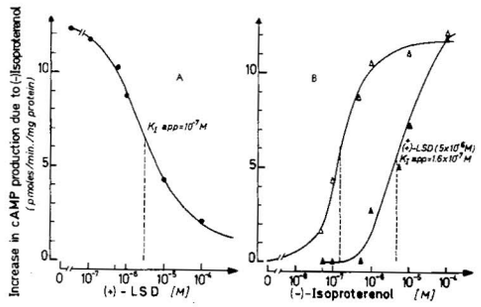

FIG 1

The effect of LSD on the (-)-isoproterenol-sensitive adenylate cyclase in homogenates of the molecular layer of the cerebral cortex of male Charles River Sprague-Dawley strain rats. Adenylate cyclase was measured as described previously (5,17), at 35°C. Each point is the mean of at least 3 independent determinations. In Fig 1A, the concentration of (-)-isoproterenol used was 5 x 10-^{6}M. The basal adenylate cyclase activity in the absence and presence of LSD (10-^{4}M)was respectively 16.2 and 15.4 pmoles/min/mg protein. In Fig 1B the dose-response curve of adenylate cyclase to (-)-isoproterenol was measured in absence (△) and presence (▲) of 5 x 10-^{6}M LSD. The basal adenylate cyclase activity in absence and presence of 5 x 10-^{6}M LSD was respectively 16.9 and 18.4 p moles/min/mg protein.

Even at high concentrations (10^{-4}M), LSD had no stimulatory or inhibitory effect on basal adenylate cyclase activity. As shown in Fig 1B, the stimulation of the β-adrenergic sensitive adenylate cyclase by LSD was competitive and the K_1 app. determined from this experiment was 1.6×10^{-7}M.

The same type of inhibition was also observed in C 6 glioma cell homogenates (Fig 2). However, whilst the affinity of the β-adrenergic receptors in this system for (-)-isoproterenol was similar to that in the cerebral cortex (2×10^{-7} M), the K_1 app. of LSD was much lower (5×10^{-6} M).

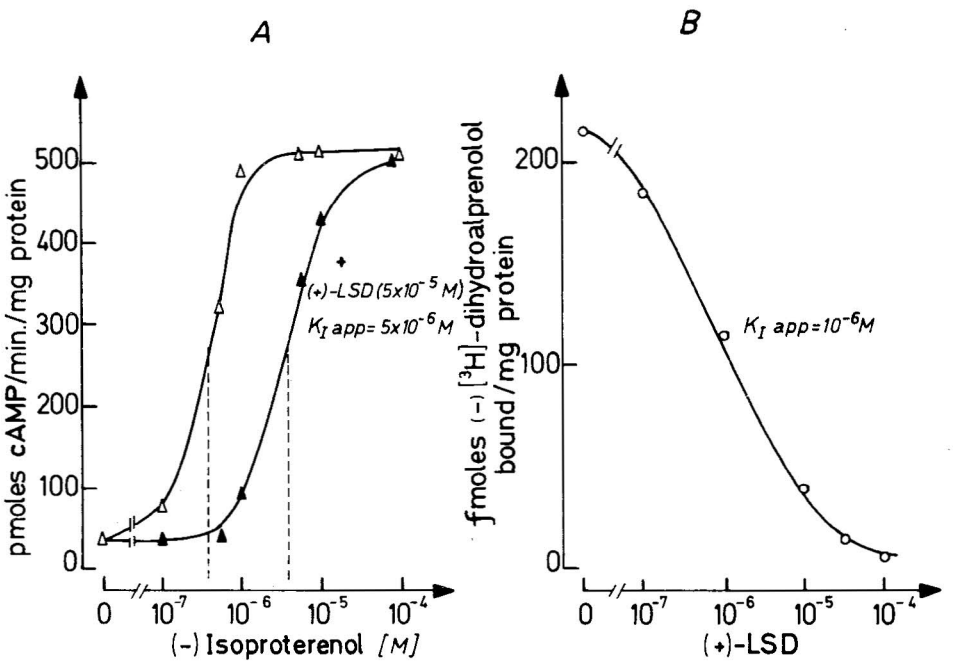

FIG 2

Effect of LSD on the β-adrenergic receptor coupled with an adenylate cyclase in C 6 glioma cells. In Fig 2A, the dose-response curve of adenylate cyclase to (-)-isoproterenol was measured in absence (△) and presence (▲) of 5×10^{-5} M LSD as previously described (18). In Fig 2B, the effect of LSD on ^{3}H-DHA (3.5×10^{-9} M) binding was determined as previously described (18).

^{3}H-DHA has previously been shown specifically to label β-adrenergic receptors (13, 14, 15). From our results, in the cerebral cortex this binding was displaced by (-)-alprenolol (K_D 1.4 x 10^{-8} M) and by (-)-isoproterenol (K_D 5.6 x 10^{-7} M). 2-bromo-(+)-lysergic acid diethylamide (BOL) was found to have the same dissociation constant as LSD in this system (Fig 3). However, at a concentration of 10^{-6} M DA, serotonin and the α-adrenergic antagonist, phentolamine did not influence ^{3}H-DHA binding ; neither did the other compounds listed in Fig 3, including the neuroleptics fluphenazine and sulpiride, and the serotonin agonists 5 M-DMT and bufotenine, and antagonists methergoline and methiothepin.

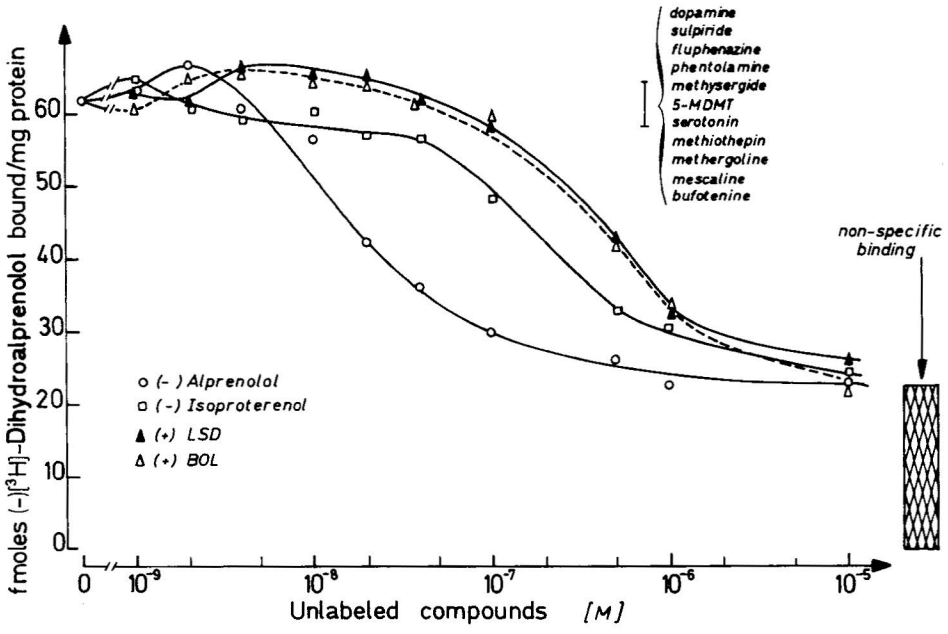

FIG 3

The relative potency of various compounds in competing for ^{3}H-DHA binding in particulate fractions of rat cerebral cortex. The cortex was homogenised in 10 volumes of ice-cold 0.05 M Tris-HCl, pH 8, diluted 6 fold and centrifuged at 50,000 g for 20 min. The pellet was washed 3 times and the final pellet was resuspended in 10 volumes of 0.05 M Tris HCl, containing 0.1% ascorbic acid and 1 μM pargyline. Samples of 90 μl (425 μg protein) of this particulate suspension were incubated at 23°C in the presence of 2.3 x 10^{-9}M ^{3}H-DHA, in a final volume of 100 μl for 15 min and were then filtered through 2 Whatman GF-C filters, and washed 5 times with 4 ml ice-cold Tris HCl 0.05 M pH 8. The ^{3}H-DHA retained on the upper filter was then estimated. All determinations were performed triplicate. Displacement of ^{3}H-DHA by (-)-alprenolol (-○-), (-)-isoproterenol (-□-), LSD (-▲-) and BOL (-△-) was measured. The displacement of ^{3}H-DHA by various other compounds (10-^{6}M) was within the limits shown, in the order given.

The inhibition of LSD of ^{3}H-DHA binding was competitive, showing in this case a K_D of 3 x 10-^{7}M (Fig 4). In addition, a study of the time course of binding of ^{3}H-DHA in the presence or absence of LSD indicated that the inhibition occured extremely rapidly, in less than 1 minute, and was reversible by washing the membrane fraction.

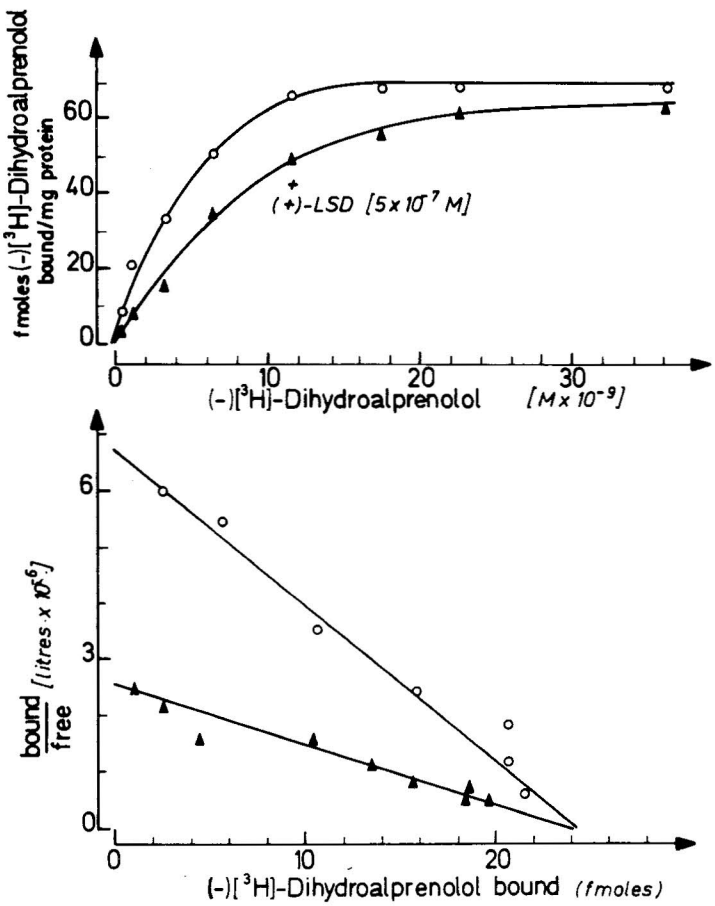

FIG 4

Competitive inhibition of ^{3}H-DHA binding by LSD. The membrane fraction, prepared as described in the legend to Fig 3, was assayed for specific ^{3}H-DHA binding using increasing concentrations of ^{3}H-DHA, in the presence (—▲—) and absence (—●—) of LSD (5 x 10-^{6} M). Specific binding at 35°C was defined as the difference in the amount of ^{3}H-DHA bound in the absence and presence of 10-^{5} M (-)-isoproterenol. The lower graph shows Scatchard plot of the results and gives a K_D of 3.6 x 10-^{9} M for ^{3}H-DHA binding. The K_D for LSD may be calculated as equal to 3 x 10-^{7} M.

DISCUSSION

It is clear from the results presented that LSD interacts with central β-adrenergic receptors coupled to adenylate cyclase. In the cerebral cortex, its affinity for both the ^{3}H-DHA binding sites and the β-adrenergic sensitive adenylate cyclase is about 10^{-7} M which is similar to the affinity of the very potent β-adrenergic agonist (-)-isoproterenol.

LSD produces marked behavioural changes when administered to rats, some of which have been attributed to interaction with central β-adrenergic receptors. For example, it has been reported that the "abberant behaviour" of rats produced by LSD is blocked by the β-adrenergic antagonist propranolol (6). In addition LSD has been found to increase noradrenaline (NA) turnover in rat brain (7) and to block the NA-induced increase in cyclic AMP in slices of rat hypothalamus (8). From these and the present results it appears that LSD is able to bind to central β-adrenergic receptors both in vivo and in vitro.

Conversely, there are several reports that certain behavioural and pharmacological serotonin agonist effects may be inhibited by β-adrenergic antagonists (10 - 12). The conclusion drawn from these studies has been that antagonists of β-adrenergic receptors may also acts as antagonists at central serotonin receptors. However, in the light of the present results this conclusion is open to question.

The affinity of LSD for β-adrenergic receptors is of the same order as its affinity for the DA-sensitive adenylate cyclase (5) and for the α-adrenergic binding sites (9), although a somewhat higher affinity is shown for the DA and serotonin binding sites (5-10 fold greater).

BOL, an LSD derivative with much weaker hallucinogenic effects in man was, however, found in this study to have the same affinity for β-adrenergic binding sites as LSD. This has also been found to be true from LSD (19), serotonin (20) and DA binding (3) studies. Indeed, the only systems where the effects of these two compounds may be differentiated are the presynaptic inhibition of the firing of raphé neurones (21) where LSD is more potent than BOL, and the striatal DA-sensitive adenylate cyclase (2) for which LSD is a partial agonist, while BOL has only antagonist properties.

Whether the psychomimetic effects of LSD may be explained solely by its action on either of these two systems remains to be elucidated. However the demonstration of the pleiotropic properties of LSD lends weight to the view that the psychomimetic action of LSD is not the result of an effect on a single type of receptor, but is rather due to the integration of simultaneous interactions with several monoaminergic neuronal systems, each one being essential.

The present results indicate that the β-adrenergic receptor is likely to comprise one of these multiple sites of action of LSD.

ACKNOWLEDGEMENTS

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