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Naloxone and Propranolol Inhibit the Disruption of the Hippocampal Theta Waves Induced by D-Lysergic Acid Diethylamide in the Rabbit

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ORTOLANI, E , G DE MARIA AND S PALAZZESI *Naloxone and propranolol inhibit the disruption of the hippocampal theta waves induced by D-lysergic acid diethylamide in the rabbit* PHARMACOL BIOCHEM BEHAV **24**(2) 183–186, 1986 —In the rabbit, naloxone and propranolol antagonize the disruption of the hippocampal theta waves induced by LSD These results are discussed in view of the reported effects of these drugs in curing hallucinatory symptoms in mentally disturbed patients

Hippocampal Theta rhythm LSD Naloxone Propranolol

IN the last few years interest was focused on the possible role played by the endorphins on the pathogenesis of acute hallucinatory syndromes and other psychiatric disturbances, an increase of β-endorphins was evidenced in the cerebrospinal fluid of acute schizophrenic patients during hallucinatory spells [16] Naloxone, which allegedly has a strong affinity for the μ receptor, can reverse and prevent hallucinatory syndromes in acute schizophrenic patients [7]

In previous investigations carried out in our laboratory [1,11], the EEG alterations observed in the rabbit after administration of lysergic acid diethylamide (LSD) were described Disruption of the theta waves of the hippocampus was considered characteristic of the EEG effects of this drug and proposed as indicative of the hallucinogenic effect in man

The aim of the present investigation was to test whether naloxone prevents or reverses the theta waves disruption induced by LSD Propranolol has been tested since this drug has a synergistic therapeutical effect with chlorpromazine in acute schizophrenics [14] and has been used in the treatment of LSD-induced anxiety states [9]

METHOD

A total of 28 adult male *Rouge de Bourgogne* rabbits weighing 2 2–2 5 kg were implanted with cortical electrodes on the sensorimotor cortex, and with bipolar electrodes in the dorsal hippocampus, under light ether plus local lidocaine anesthesia One hour following surgery the animal was placed in a restraining box, and the EEG was recorded on paper and on a magnetic tape according to the following schedule The pre-drug control EEG was recorded for one hour, during this period the animal was awakened by applying an arousing noise at regular intervals After treatment, the EEG was recorded for three hours, applying the arousing noise at the same intervals

Upon termination of the experimental session, artifact-free hippocampal EEG samples of the duration of about 2 min were chosen upon visual inspection, band-pass filtered (0 5 to 32 c/sec) and fed in a minicomputer for quantitative analysis These samples concerned records taken immediately after the arousing stimulus Sampling frequency was 128 points/sec over periodograms of 8 sec, power spectra from 0 5 to 32 c/sec were built using standard FFT algorithm, and stored on digital tape Finally, the power of strongly inter-related adjacent lines were cumulated on the basis of a procedure described previously [10] The computer printout was therefore referred to 4 frequency bands (1–4, 5–6, 7, 8–32 c/sec) for each temporal period of 2 min Power was expressed as percent of predrug total power in each animal The following drugs were used LSD (2–64 μg/kg), in 16 animals), naloxone (0 4–1 mg/kg, in 6 animals), propranolol (0 4–1 mg/kg, in 6 animals) All figures refer to the weight in base of the drugs

The differences among EEG power values had been computed using ANOVA test

RESULTS

Effects of LSD on Computerized Hippocampal EEG

Power values of 1–4 and 5–6 c/sec bands were not consistently affected by low doses (2 and 4 μg/kg), while 16 and 32 μg/kg induced a dose-related enhancement The power of 7 c/sec frequency band was consistently enhanced by 4 and 16

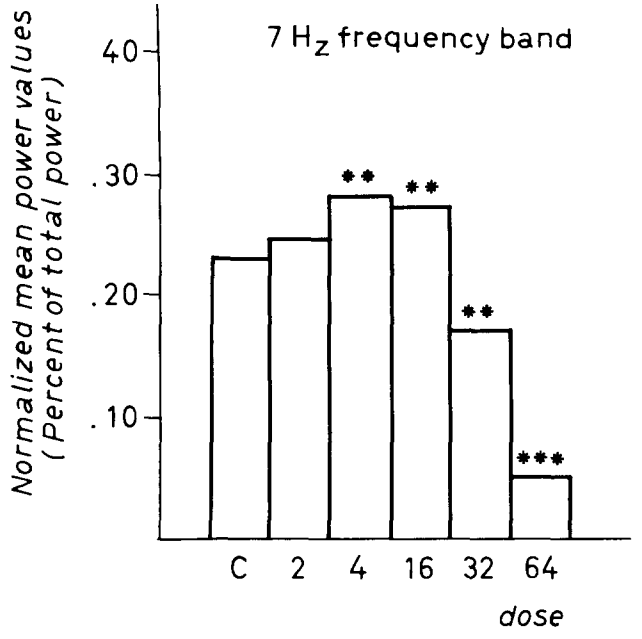

FIG 1 Effects of LSD on the EEG power values relative to dorsal hippocampus Dose-effect relationship relative to 7 Hz frequency band 15 minutes after IV injection Abscissa doses in μg/kg IV Ordinates Normalized mean power values (4 animals per dose) Significance of ANOVA versus saline (C), $^{**}p<0.01$, $^{***}p<0.001$

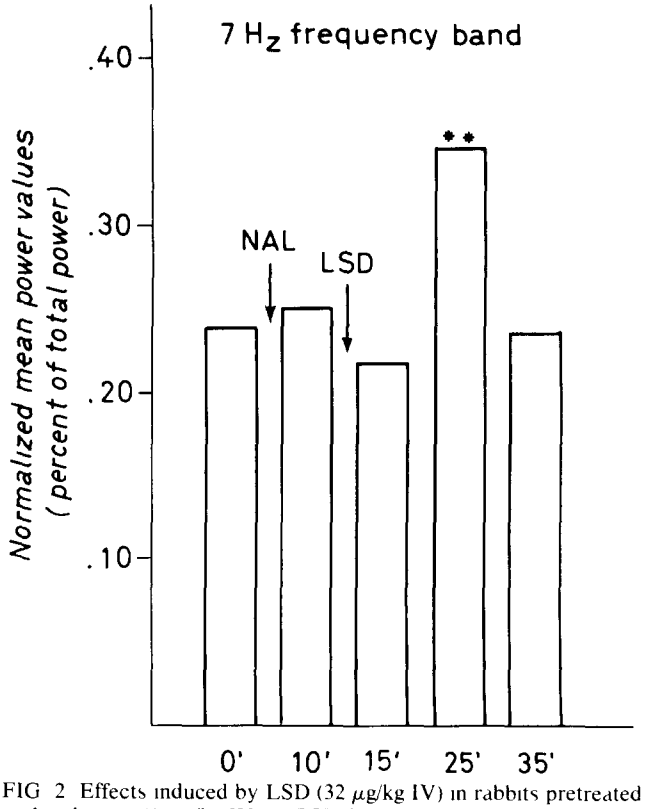

FIG 2 Effects induced by LSD (32 μg/kg IV) in rabbits pretreated with naloxone (1 mg/kg IV) on 7 Hz frequency band Abscissa time in minutes Ordinates Normalized mean power values (four animals) ANOVA significance versus predrug control $^{**}p<0.01$

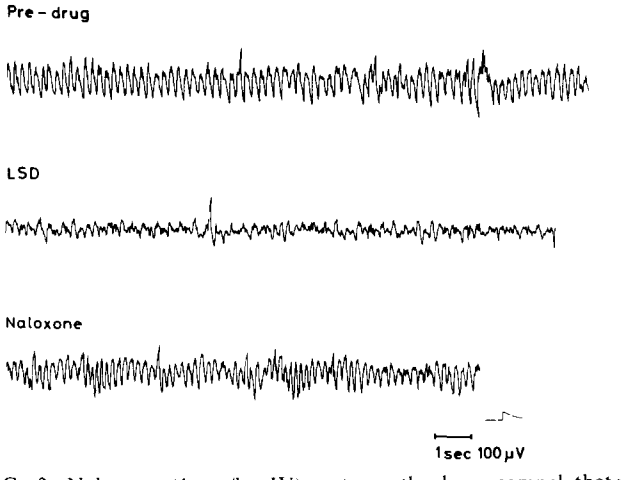

FIG 3 Naloxone (1 mg/kg IV) restores the hippocampal theta rhythm disrupted by LSD (32 μg/kg IV)

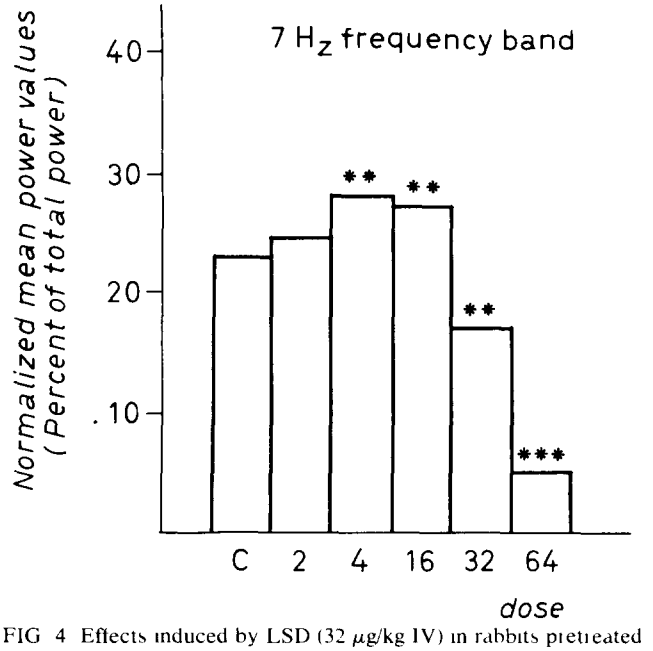

FIG 4 Effects induced by LSD (32 μg/kg IV) in rabbits pretreated with propranolol (0 4 mg/kg IV) on 7 Hz frequency band of hippocampal EEG power spectrum Abscissa time in minutes Ordinates Normalized mean power values (mean of four animals) ANOVA significance versus predrug control values, $^{**}p<0.01$ $^{***}p<0.001$

μg/kg, the dose of 32 and 64 μg/kg induced a fall of power lasting 20–30 min followed (for 32 μg/kg) by a progressive rise to control values or to values higher than controls (Fig 1) In the 8–32 c/sec frequency band an increase of power took place shortly following 2, 4 and 16 μg/kg, 32 and 64 μg/kg caused a drop of power values, return to control values was observed after 60 min

Antagonistic Effects of Naloxone on LSD

A small increase in theta waves voltage accompanied by a slight behavioral excitation was noticed after naloxone 1 mg/kg LSD (32 μg/kg), administered 5 min after naloxone, did not elicit the typical theta disruption Computerized analysis confirmed such data and evidenced, after a transitory drop, an increase of 7 c/sec power and of higher frequency bands (8–32) 15 min following LSD (mean of 4 animals) (Fig 2)

The same dose of naloxone administered 5 min following 32 μg/kg of LSD brought back to normal the theta rhythm (Fig 3) Computerized analysis confirmed this effect both with reference to the 7 Hz and to the 8–32 Hz frequency bands

Antagonistic Effects of Propranolol on LSD

Propranolol (0 4 and 1 mg/kg) did not give rise to evident behavioral or EEG changes LSD (32 μg/kg) injected 10 min following propranolol did not alter the theta rhythm under visual EEG inspection in 4/4 animals pretreated with 0 4 mg/kg of propranolol and in 2/2 pretreated with 1 mg/kg Computerized analysis showed that the power relative to 7 c/sec frequency was remarkably increased following propranolol administration and that such an increase was strengthened by LSD (Fig 4) Also the power of the higher EEG frequency bands, not modified by propranolol, increased after administration of LSD On the other hand the drug (up to 1 mg/kg) was ineffective on the theta disruption induced by a previous injection of 32 μg/kg of LSD

DISCUSSION

LSD induces disruption of hippocampal theta waves in the rabbit this phenomenon is accompanied by the disappearance of the single pyramidal cell activity recorded with microelectrodes [2] A similar effect on the EEG and on single-unit activity was described for tryptamine [1,11] These data suggest that the serotoninergic system plays an inhibitory role on the pyramidal cell firing through a mechanism not yet clarified The fact that naloxone prevents or reverses the LSD-induced theta disruption indicates a possible involvement of the opioid system in the genesis of these alterations This hypothesis receives confirmation from clinical data (see Introduction) and from the fact that the psychotomimetic action induced in man by opiate agonists such as pentazocine, cyclazocine, levallorphan, cyclorphan can be also antagonized by naloxone [8] Fertziger and Fisher [4] described an antagonism between naloxone and LSD in the rat, on the basis of the "analgesic bliss" observed for LSD and other hallucinogenic substances, these authors mention the possibility of an interaction of these drugs with the endorphinergic system

Several data indicate that propranolol is endowed with central effects For this drug improvement of anxiety states has been described, together with beneficial effects in drug dependence [5,6] and in essential tremor [15] The mechanism of action underlying these activities is still obscure, but theories about mode of action of this drug have been already advanced, including β-adrenergic blockade, cell membrane stabilization, and 5-HT receptors blocking action [12] Our data support this last hypothesis and confirm other results found in the literature Propranolol antagonizes the head twitches induced by 5-hydroxytryptophan in mice, or 5-HT sleep in young chicken [17] Biochemical investigations have indicated that the potency of propranolol in blocking 5-HT binding is equal or greater than its potency against LSD binding [3, 12, 13] This competitive antagonism toward common receptorial sites could be responsible for the prevention of the EEG effects induced by LSD However, it can be supposed that this competitive activity is not sufficiently strong to displace LSD once is already binded to the receptor

REFERENCES

1 Baran, L and V G Longo Instrumental reward discrimination in rabbits Electroencephalographic and behavioral effects of a series of lysergic acid derivatives *Acta Physiol Latinoam* **14**, 125–137, 1964

2 Brucke, F G Gogolak and C H Stumpf Die Wirkung von LSD and die Makround mikrotatigkeit des hippocampus *Arch Exp Pathol Pharmakol* **240**: 461–468 1961

3 Connell D J D N Middlemiss and M A Stone Further evidence for an interaction of propranolol with the central 5-hydroxytryptamine (5-HT) receptor Proceeding of the B P S 12th–14th September, 173–174P, 1979

4 Fertziger, A P and R Fisher Interaction between narcotic antagonist (Naloxone) and Lysergic Acid Diethylamide (LSD) in the rat *Psychopharmacology (Berlin)* **54** 313–314, 1977

5 Granville-Grossman, K L and P Turner The effect of propranolol on anxiety *Lancet* **1** 788–790 1966

6 Grosz, H J Successful treatment of heroin addict with propranolol Implications for opiate addiction treatment and research *J Ind State Med Assoc* **65**: 505–509, 1972

7 Gunne, L M L Lindstrom and L Terenius Naloxone-induced reversal of schizophrenic hallucinations *J Neural Transm* **40**: 13–14, 1977

8 Jasinski, D R , W R Martin and J D Sapira Antagonism of the subjective behavioral pupillary and respiratory depressant effects of cyclazocine by naloxone *Clin Pharmacol Ther* **9**: 215–222, 1968

9 Linken, G A Propranolol for LSD-induced anxiety states *Lancet* **6**: 1038–1040, 1971

10 Loizzo, A , G A Zapponi, A Giuliani and V G Longo Computer assisted electroencephalographic monitoring of pesticides effects in animals In *Adverse Effects of Environmental Chemical and Psychotropic Drugs*, vol 2, edited by E Horvath Amsterdam Elsevier, 1976, pp 279–283

11 Longo, V G *Neuropharmacology and Behavior* San Francisco W H Freeman, 1972

12 Middlemiss, D N , L Blakeborough and S R Leaker Direct evidence for an interaction of β-adrenergic blockers with the 5-HT receptor *Nature* **267**: 289–290, 1977

13 Middlemiss, D N , A Buxton and D T Greenwood β-Adrenoceptor antagonists in psychiatry and neurology *Pharmacol Ther* **12**: 419–437, 1981

14 Peet, M Is propranolol antischizophrenic? *Neuropharmacology* **20**, 1303–1307, 1981

15 Sevitt, I The effect of adrenergic beta-receptor blocking drugs on tremor *Practitioner* **207**: 677–678, 1971
16 Van Ree J M and D De Wied Endorphins in schizophrenia *Neuropharmacology* **20**, 1271–1275, 1981
17 Weinstock, M , C Weiss and S Gitter Blockade of 5-hydroxytryptamine receptors in the central nervous system by β-adrenoceptor antagonists *Neuropharmacology* **16**: 273–276, 1977