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Hapten-Immunological Studies on Mescaline

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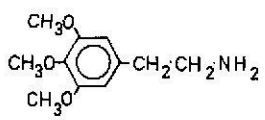
Abstract. Antibodies with mescaline binding specificity were raised in rabbits by immunization with conjugates of bovine serum albumin with mescaline or its analogue 3,4,5-trimethoxyphenylacetic acid. Immunized rats were given mescaline and their behavior was compared to that of non-immunized controls.

Key words: Mescaline – Anti-Drug Antibody – Hapten – Immunopharmacology – Rabbit – Rat.

During the past decade it has become obvious that immunology offers a series of potent and rather simple methods of great interest for other spheres of biological research. One of these is the ability of mammals to synthesize antibodies with binding capacity for several drugs (Gross *et al.*, 1968; 1971; Grant *et al.*, 1972; Rubenstein *et al.*, 1972; Sher *et al.*, 1973).

The present investigation shows the possibility of inducing antibodies against mescaline (3,4,5-trimethoxyphenethylamine, I, designated Mes*) and the structurally related 3,4,5-trimethoxyphenylacetic acid (II, designated Mes') in rabbits and rats. The structural parts of the haptens determining the binding specificity were identified by submitting the antisera to cross-inhibition experiments with related compounds.

In addition, a contribution to the field of pharmacopsychiatry was attempted. The effect of immunization against mescaline on drug induced behavioral alterations of experimental rats was studied. Passive immunization to abort acute drug effects, as a possible alternative to the unsatisfactory antagonist chlorpromazine (Schmidbauer, 1971, p. 149; Shah and Green, 1973), was also considered.



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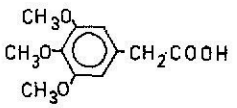

II

Methods

Reagents. Mescalinium chloride was purchased from Sigma (St. Louis, Mo., U.S.A.). 3,4,5-trimethoxy (345-TMO) phenylacetic acid, 345-TMO cinnamic acid, 345-TMO, 246-TMO, 3,4,5-triethoxybenzoic acid, gallic acid (3,4,5-trihydroxybenzoic acid, monohydrate) pure grade were from Fluka AG (Buchs, Switzerland).

The proteins used were bovine serum albumin (BSA) "Pentex" BV 0262 (Fluka) and bovine gammaglobulin (BGG) Cohn fraction II (Sigma).

The coupling reagent was N'-(3-dimethylaminopropyl) carbodiimide · HCl (ECDI) (pure grade, Fluka).

Buffer. Phosphate buffered saline (PBS), 0.14 M NaCl, 0.01 M phosphate pH 7.2, was used.

Coupling of Mes' and Mes to BSA and BGG.* Carboxyl and amino groups were coupled with the soluble carbodiimide ECDI as described by Spector and Parker (1970).

1. Mes'-BSA: 40 mg BSA [60 lysine residues per molecule (Keil and Šormová, 1965)] dissolved in 10 ml of water were mixed with 80 mg of Mes' (10 fold molar excess) in 15 ml of water. The pH was adjusted to 5.5 (1 N NaOH) and 20 mg of ECDI freshly dissolved in 4 ml of water were added. The solution was stirred for 20 hrs at room temperature and then dialysed under flowing tap water for 40 hrs and against PBS for 24 hrs.

2. Mes*-BSA: 18 mg of BSA and 40 mg of Mes* in 8 ml of water. The pH was raised to 12.15 with 0.1 N NaOH and 20 mg of ECDI in 4 ml of water were added. The other manipulations were as above.

3. Mes'-BGG: 45 mg of BGG and 43 mg of Mes' were dissolved in 20 ml of PBS. 40 mg of ECDI were added in solid form. The mixture turned turbid and was stirred for 24 hrs at room temperature. After centrifugation

(3000 × g, 15 min) the sediment was suspended in PBS and stirred for 15 hrs. The supernatant contained conjugate.

Determination of Coupling Ratio. The amount of Mes' groups per carrier molecule was estimated by their absorption maxima at 268 and 278 nm. Another method was ultracentrifugal comparison of the sedimentation velocity of free BSA and conjugate (Beckman Model E analytical ultracentrifuge, standard equipment, wedge cell, schlieren optics). The sedimentation increment was evaluated with a graphical procedure.

Animals. Rabbits were locally purchased. 6 male DA rats from the same litter had an age of 5 weeks at the beginning of the immunization procedure.

Immunization. The highest titer of anti-hapten antibody in rabbits was reached by intramuscular injection of 1.2 ml of Mes'-BSA (1 mg/ml) and Mes*-BSA (1.7 mg/ml) respectively in PBS emulsified with 1.2 ml of complete Freund's adjuvant into the gluteal muscles at three week's intervals. The sera were harvested three weeks after the fifth injection.

DA rats were immunized with the same emulsion of Mes'-BSA by the intraperitoneal route every two weeks. The volumes applied were 0.2, 0.4, 0.4, 0.5, 0.5, 0.5, 0.5, ml. *In vivo* experiments were performed 5 weeks after the last injection, the sera beeing tested for anti-mescaline activity.

Absorption. For some experiments the anti-carrier activity in anti-Mes'-BSA antisera was eliminated. Empirically determined amounts of BSA were added. The precipitates were removed by centrifugation (3000 × g, 15 min) after 20 hrs. The sera will henceforth just be designated "absorbed".

Immuno Assays. Antigen antibody reactions were made visible with the double diffusion technique of Ouchterlony (1958).

Haptenic inhibition of the antibody activity was shown by a variant of Ouchterlony assay. Antisera were mixed with equal volumes of neutralized 0.5 % solutions of the haptens to be tested. After 3 hrs the solution was diffused against 0.1 % solution of Mes'-BSA in PBS.

Effect of Mescaline in Immunized and Native Rats. The minimum quantity of a 3 % solution of mescalinium chloride in PBS necessary for significant behavioral alterations was determined empirically. Three quantities near this minimum were given intraperitoneally and the time course of alterations was observed.

Results

Coupling Ratios. 30–50 Mes' groups were bound to BSA as determined spectrally. Sedimentation analysis indicated a coupling ratio of 43:1.

The amount of Mes' linked to BGG was about 0.1 mg/mg, which is similar to that bound by BSA.

The coupling ratio of Mes* to BSA was not determined.

Specificity of Antisera. Both unabsorbed and absorbed antisera cross-reacted with Mes'-BGG conjugate (Figs. 1, 2).

Absorbed antiserum did not cross-react with BSA (Fig. 3).

Mes'-BSA and Mes*-BSA developed against absorbed anti-Mes'-BSA from neighbouring wells showed a pattern of partial identity, probably due to different distribution of the two haptens on the surface of BSA (Fig. 4).

The precipitating power of anti-Mes'-BSA was completely extinguished by Mes' and Mes* (Fig. 5). The antibody activity was partly recovered after treatment with 345-TMO benzoic acid and 345-TMO cinnamic acid (Fig. 6). It was not influenced by 246-TMO, 3,4,5-triethoxybenzoic acid, *l*-adrenaline, and gallic acid.

Mes'-BSA developed against anti-Mes'-BSA and anti-Mes*-BSA alongside formed a spur towards the anti-Mes*-BSA reservoir depicting the difference in specificity of the two antisera and therefore in the structure of the two immunogenic conjugates (Fig. 7).

Influence of Anti-Mescaline Upon Drug Effect in Rats. 13 mg of mescalinium chloride injected i.p. produced a definite effect in DA rats (300 g body weight):

1. Breathing frequency increased with the strength of the effect (Jochimoglu and Keeser, 1924) and was used as a quantitative measure.

2. "Crawling" was a symptom during the most acute phase. The animals moved about their cages with stretched-out hind legs.

Fig. 8 compares the effect of mescaline (13.5, 14.5, 15.0 mg) in normal and immunized rats and shows the apparently milder and more lasting course in terms of their breathing frequency and crawling in immunized animals.

The absorbed rat sera displayed an anti-Mes'-BSA reaction comparable to that of rabbits (Fig. 9).

Discussion

The cross-inhibition experiments with small molecules of similar structure to Mes' showed the specificity of absorbed anti-Mes'-BSA to be directed mainly against the aromatic part of the molecule, which possesses a rigid configuration (Karush, 1962), rather than against the alkyl side chain, with which Mes' was bound to the protein. As was to be suspected the surrounding region of the carrier around the haptenic ligands accounted for part of the antibody specificity.

In immunized rats the anti-mescaline antibody seemed to react as a depot for the administered drug. As mescaline is a weak hallucinogen [8000-fold molar amount of LSD for minimm hallucinating experience in humans (Schmidbauer, 1971, p.109)], huge amounts of antibody would have been necessary for quantitative binding of the injected mescaline. For this

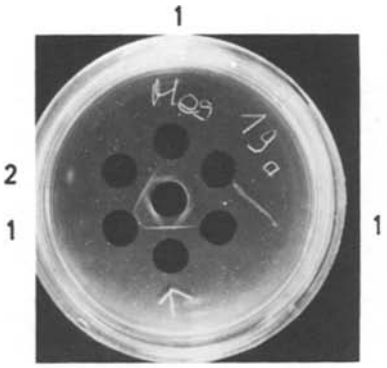

Fig. 1

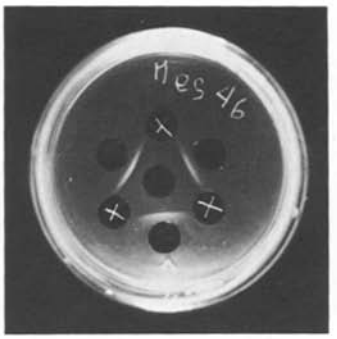

Fig. 2

Fig. 1. Precipitation of Mes′-BSA and Mes′-BGG by anti-Mes′-BSA. *1* Mes′-BSA; *2* Mes′-BGG; center = anti-Mes′-BSA

Fig. 2. Precipitation of Mes′-BGG by absorbed anti-Mes′-BSA. Periphery – anti-Mes′-BSA absorbed; center = Mes′-BGG

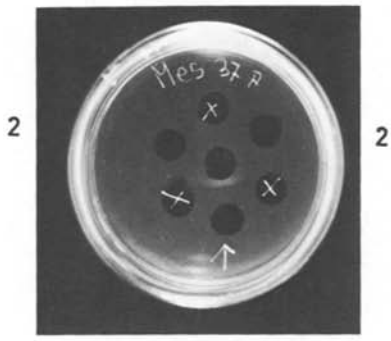

Fig. 3

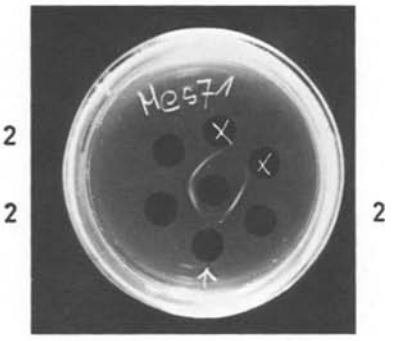

Fig. 4

Fig. 3. Absorbed anti-Mes′-BSA does not cross-react with BSA. *1* Mes′-BSA; *2* BSA; center = anti-Mes′-BSA absorbed

Fig. 4. Spur formation of Mes′-BSA and Mes′-BGG against absorbed anti-Mes′-BSA. *1* Mes′-BGG; *2* Mes′-BSA; center = anti-Mes′-BSA absorbed

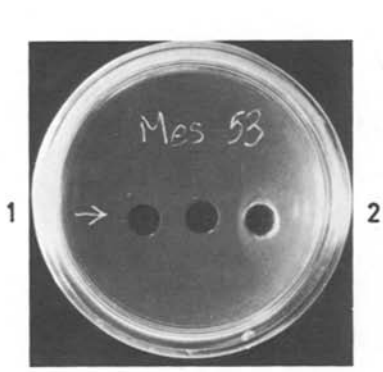

Fig. 5

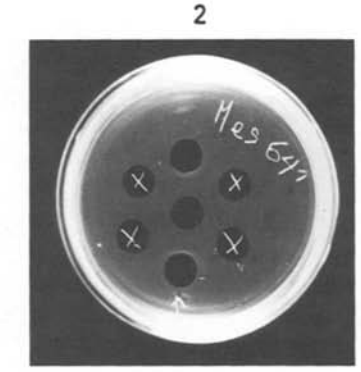

Fig. 6

Fig. 5. Hapten inhibition of anti-Mes′-BSA with Mes*. *1* anti-Mes′-BSA + Mes*; *2* anti-Mes′-BSA; center = Mes′-BSA

Fig. 6. Partial inhibition of anti-Mes′-BSA by two mescaline analog compounds. *1* anti-Mes′-BSA + 345-TMO benzoic acid; *2* anti-Mes′-BSA + 345-TMO cinnamic acid; center = Mes′-BSA

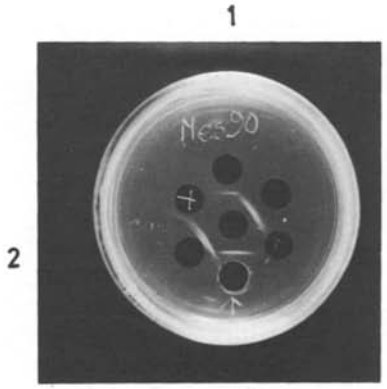

Fig. 7

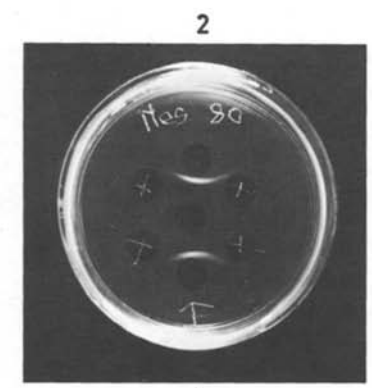

Fig. 9

Fig. 7. Spur formation between anti-Mes′-BSA and anti-Mes*-BSA developed against Mes′-BSA. *1* anti-Mes*-BSA; *2* anti-Mes′-BSA; center = Mes′-BSA

Fig. 9. Anti-Mes′ activity in two immunized DA rats. *1, 2* absorbed sera; center = Mes′-BSA

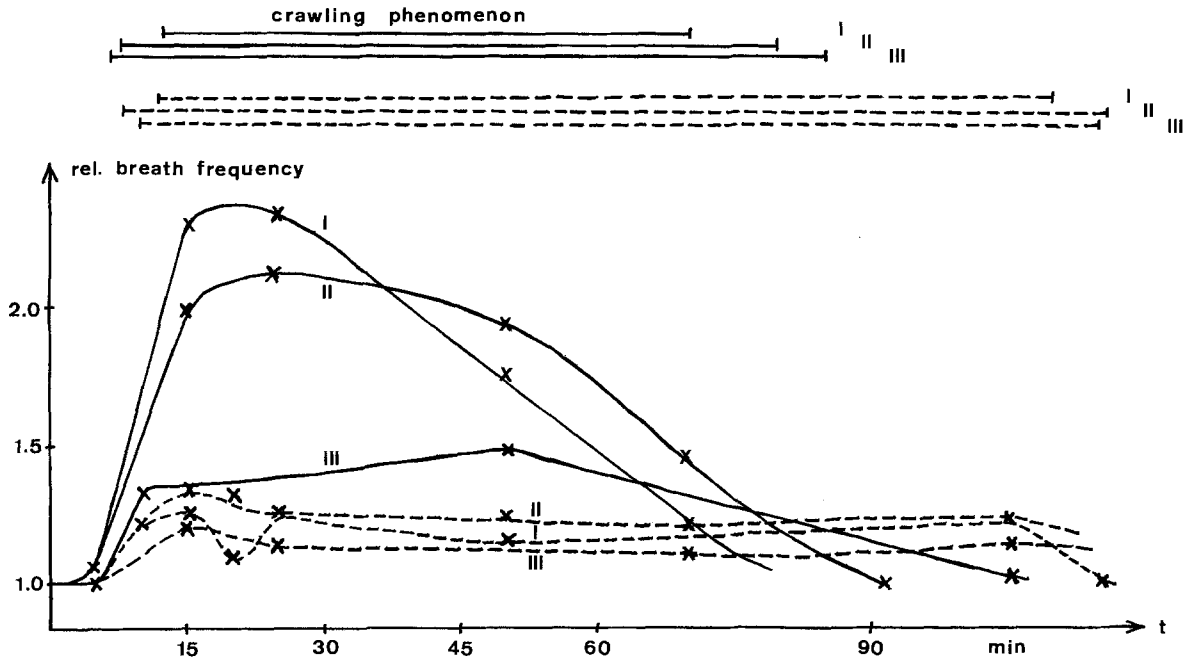

Fig. 8. Mescaline alterations in immunized and normal DA rats. —— normal; ----- immunized DA rats. *I* 13.5 mg, *II* 14.5 mg, *III* 15.0 mg mescaline

reason only partial inhibition of the mescaline effects could be observed. The ability to interrupt tripping experience by passive administration of anti-drug antibody should therefore be established on a strong hallucinogen such as LSD, against which antibodies have been induced by Sher *et al.* (1973).

Vaccination against abuse of marijuana and hashish, which are drugs with a low dose effect (vom Scheidt, 1971), with a conjugate of THC and a carrier protein (Grant *et al.*, 1972) seems feasible.

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