Specific in vivo binding of d-LSD in rat brain

K. Kräuchi, H. Feer, M. Lichtsteiner and A. Wirz-Justice[1]

Psychiatrische Universitätsklinik, Wilhelm-Klein-Strasse 27, CH-4025 Basel (Switzerland), 2 Dezember 1977

Summary. A reproducible in vivo d-LSD binding method in rat brain is described, with high affinity (K_d of 5 pmoles/g wet wt), stereospecificity (d- vs. l-LSD) and regional selectivity. It may be a useful adjunct to in vitro methods for measuring changes in turnover at the synaptic level related to the intact receptor.

The in vitro binding of d-LSD to synaptic membrane preparations from rat brain involves both serotoninergic and dopaminergic receptors[2,3]. Despite their widespread usefulness, such in vitro investigations have one critical disadvantage: preparation of the membranes disrupts the endogenous ionic balance and perhaps also the functional state of the receptor in its immediate environment. In vivo binding is similar to in vitro[4,5], and has the advantage of being able to measure a biological or pharmacological effect on the receptor in its physiological state. We investigated whether the in vivo method was also applicable to d-LSD binding.

Experimental. Adult male albino Wistar rats (180–220 g) were kept under controlled conditions of L:D 12:12, temperature 24 °C, and feeding ad libitum. The experiments were carried out in the middle of the light phase (12±2 h). Tritium-labelled d-LSD (Amersham, 11.1 Ci/mmole, 1.5 µg/kg in 200 µl 0.1% ascorbic acid) was injected i.v. via the tail vein. The rats were decapitated 30 min after injection and their brains rapidly dissected on ice into cerebellum (CE), cortex (CO), striatum (ST), medulla and pons (MP), thalamus and hypothalamus (HT) and hippocampus (HI)[6]. Each region was homogenized in Tris buffer (0.05 M, pH 7.7, 3 ml) and aliquots (0.5 ml) shaken with Soluene (0.1 ml) and Instagel (10 ml: both Packard). The samples were counted for 10 min in a Packard Tricarb scintillation counter with an efficiency of 32% that was constant and not corrected. Increasing doses of cold d- or l-LSD were simultaneously injected i.v. with ^{3}H-d-LSD in displacement experiments; other drugs were injected i.p. in 500 µl physiological saline at different times before i.v. injection of ^{3}H-d-LSD.

Results and discussion. Total ^{3}H-d-LSD binding showed reproducible regional differences, being highest in striatum and lowest in cerebellum (figure 1). As in vitro[7,8], dose-dependent displacement by unlabelled d-LSD was found in vivo, again most prominently in the striatum (figure 2). Scatchard plot analysis of the d-LSD binding data from figure 2 revealed that maximally 20 pmoles/g wet wt could be bound in the striatum, with a dissociation constant K_d of ca. 5 pmoles/g wet wt. This K_d in vivo is 10^2 times less than that calculated for in vitro studies[7] and may be related to the intactness of the receptor site in vivo. (According to Bennett and Snyder[7], 10 mg tissue/ml incubation medium

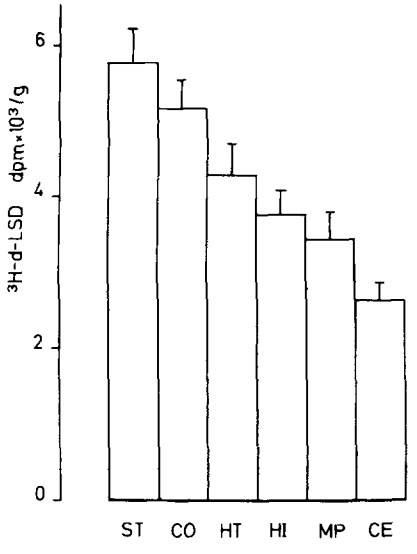

Fig. 1. Regional distribution of total ^{3}H-d-LSD binding in rat brain, 30 min after i.v. injection (mean ±SEM of 6–8 determinations).

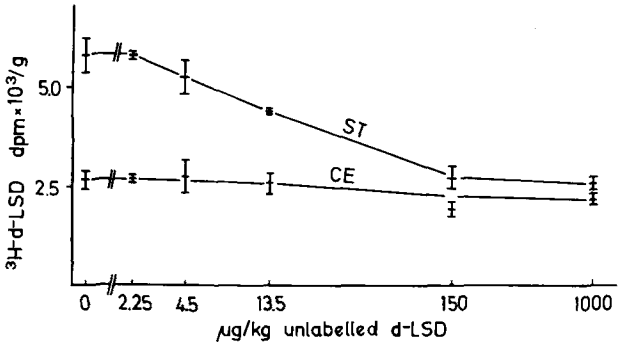

Fig. 2. Displacement of ^{3}H-d-LSD from striatum and cerebellum of rat brain by unlabelled d-LSD. Each point represents the mean ±SEM of 3–6 measurements.

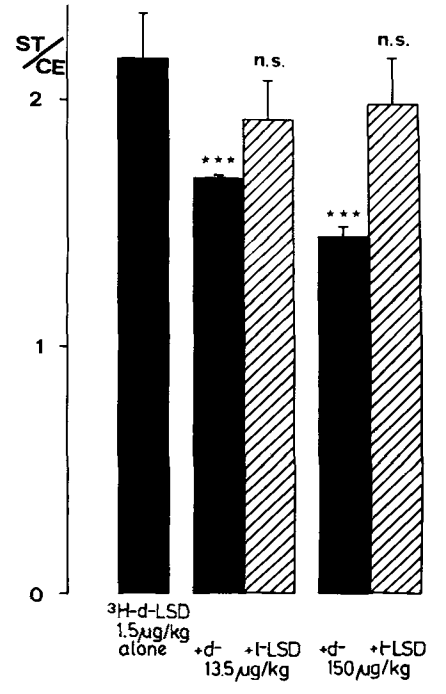

Fig. 3. Stereospecificity of ^{3}H-d-LSD binding: comparison of displacement by d- and l-LSD at 2 different concentrations (mean ±SD of 3–6 determinations, Student's t-test, ^{3}H-d-LSD vs cold l- or d-LSD, ***$p < 0.001$, n.s. $p > 0.05$).

were used, and a K_D of 6 nM found in striatum. Calculation of this in vitro K_D in terms of wet wt gives a K_D of 600 pmoles/g.) In the cerebellum, a negligable amount of saturable d-LSD binding was found. This allows the use of the cerebellum as an 'internal standard'[5] for the amount of unspecifically bound d-LSD. By calculating the ratio of the amount of specifically bound d-LSD in a given region to the amount of unspecifically bound d-LSD in the cerebellum, a correction for the experimental procedure and day-to-day fluctuations is obtained. This correction results in an interindividual variation coefficient of less than 10%, smaller than that obtained in vitro, and testifies to the remarkable reproducibility of the method.

The stereospecificity of d-LSD binding was demonstrated by the fact that specific binding could be reduced by the d- but not the l-enantiomer of unlabelled LSD injected simultaneously (figure 3), even at the relatively high dose of 150 µg/kg.

Preliminary studies of d-LSD displacement with a number of drugs were carried out using the maximum displacement found with 1 mg/kg cold d-LSD as 100%. The mean percent displacement for all regions was similar for a given drug. In striatum, neither apomorphine (5 mg/kg 60 min before ^{3}H-d-LSD; displacement 18±14% [SD, N=3]) nor 1–5HTP-ethylester (100 mg/kg 120 min before ^{3}H-d-LSD; displacement 5±13%) had a noticeable effect, whereas haloperidol (1.25 mg/kg 90 min before ^{3}H-d-LSD; displacement 63±9%) and methiothepin (20 mg/kg 120 min before ^{3}H-d-LSD; displacement 92±10%) had marked effects. Without extensive dose-response curves, no conclusion as to the relative efficacy of dopamine and/or serotonin agonist/antagonists on d-LSD binding can be drawn; however, such pharmacological studies in vivo, in particular by improving the dissection technique to measure d-LSD binding in specific dopaminergic or serotoninergic brain nuclei (in preparation) may provide more detailed information.

This method of d-LSD binding in vivo is therefore characterized by high affinity, stereospecificity and regional selectivity. It may be a useful adjunct to in vitro methods for measuring changes in turnover at the synaptic level. Its advantage over in vitro methods is the intact environment of the receptor at the time of injection.

1 We thank Sandoz AG Basel for the generous gifts of d- and l-LSD, and Hoffman-La Roche Laboratories Basel for methiothepin and 1–5HTP-ethylester.
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Bacillus thuringiensis δ-endotoxin: Evidence that toxin acts at the surface of susceptible cells

P.G. Fast, D.W. Murphy and S.S. Sohi

Forest Pest Management Institute, Department of Fisheries and the Environment, Sault Ste. Marie (Ontario P6A 5M7, Canada), 5 December 1977

Summary. Enzymically activated δ-endotoxin of *Bacillus thuringiensis* covalently bound to Sephadex beads, has the same effect on insect cells in tissue culture as free toxin. The effect is prevented by antitoxin antibody and heat denaturation and is not due to a nonspecific protein effect, the beads, or toxin released from the beads. The toxin, therefore, probably acts at the cell surface.

The insecticidal activity of *Bacillus thuringiensis* preparations resides principally in the proteinaceous parasporal inclusion (δ-endotoxin) formed as a concomitant of sporulation[1]. The parasporal body is dissolved in the insect gut to yield toxic fragments ranging in size from <5,000 to >100,000 daltons[2]; however, only atoxic dipeptide fragments of toxin are detected in hemolymph after ingestion of toxin[3]. The direct effect of the toxin appears to be limited to the epithelial cells of the midgut which suffer metabolic disturbances[4], apical swelling, and lysis[5]. Death of the insect appears to stem from leakage of gut contents into the hemocoel due to loss of integrity of the gut wall[6].

Toxin stimulates glucose uptake by midgut epithelium within 1 min after oral administration[4]. It does not, however, cause accumulation of Na^+, K^+, Ca^{++} or Mg^{++} ions[7] as had been suggested[2]. We recently showed that some insect cell lines in tissue culture are susceptible to the toxin with an LD_{50} of 0.06 mg/ml[8]. With immobilized toxin we could determine if the toxin acts at the cell surface or needs to be ingested by the cell.

Materials and methods. Enzyme-digested toxin labelled with L-leucine-H^3 was prepared as previously described[8]. The digest containing the activated toxin in 0.05 M cyclohexylaminopropane sulfonic acid (Caps) buffer pH 10.5 was passed through a 2.5×25 cm column of Sephadex G-50 medium equilibrated in the same buffer. Excluded peptides were pooled and refrigerated until required.

1 g Sephadex G-25 fine beads were swollen and activated with cyanogen bromide as described for agarose beads by Cuatrecasas and Anfinsen[9]. The activated beads (~4 ml) were washed with 200 ml Caps buffer pH 10.5 and then added to the pooled excluded peptides from the G-50 column and gently rocked overnight at 23 °C. Unbound peptide was removed by filtration and the beads washed with 200 ml Caps buffer followed by 50 ml of buffered insect saline (BIS)[8].

Assays were conducted by adding 0.1 ml of test material in BIS to 0.1 ml of BIS containing 1.5–2.0×10^5 Cf-124 cells[10] and incubating for 30 min at 28 °C. The viable cells remaining at the end of the period were detected by measuring the residual ATP. The ATPases normally released by dead cells would rapidly hydrolyze any ATP free in solution. They were destroyed, and the incubation terminated, by adding 2 ml boiling 0.05 M Tris buffer pH 7.7 and boiling for an additional 10 min. ATP was determined by the luciferin-luciferase reaction[11]. Each experiment included untreated controls and maximum response controls determined with unbound toxin. The percentage reduction in ATP compared to the untreated control was used as an estimate of the percentage reduction