This is surprising in view of the fact that precisely this was observed when 5-HT transmission was impaired by means of receptor blockade[4]. It seems conceivable that adaptive phenomena developing during the 2 week's PCPA treatment might be responsible, although experimental evidence for this is lacking.

The principal result of this study, however, was that potentiation of the effects of haloperidol on DA metabolism by drugs increasing 5-HT transmission and their antagonism by drugs reducing it were observed in the projection areas of the 3 main dopaminergic systems of the brain. Although there were some differences in the statistical significance of these effects from one area to another, there is no reason to assume that there are fundamental differences in their extent. If they are, in fact, due to functional interactions between 5-HT and DA, the serotoninergic modulation of dopaminergic transmission must presumably be a general phenomenon in the brain.


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## In vivo $^3$H-d-LSD binding in small punched out rat brain regions


K. Kräuchi and H. Feer[1]

*Psychiatrische Universitätsklinik, Wilhelm-Klein-Strasse 27, CH-4025 Basel (Switzerland), 21 December 1979*



*Summary.* We have developed an in vivo $^3$H-d-LSD-binding method in the rat brain to measure the $^3$H-d-LSD concentration in specific brain regions and nuclei. In the cortex, d-LSD binding sites increase from the occipital to the frontal part without a change in their affinity. Compared to the hippocampus, striatum, cortex and raphe nuclei, the substantia nigra contains a d-LSD binding site of lower affinity.


In contrast to in vitro methods, the binding of d-LSD in vivo is not disturbed by a change in the environment or a disruption of membranes. The binding sites remain in a physiological state and in their natural surroundings. As the in vivo method is very sensitive[2] it seemed that it would be possible to compare the affinity for d-LSD of small brain regions and single nuclei and to gain insight into the pattern of specific d-LSD binding within the brain. We therefore developed an in vivo $^3$H-d-LSD binding method with subsequent isolation of small brain regions by a punch-out technique. Neurochemical, electrophysiological and behavioural studies have shown that d-LSD binds preferentially to serotoninergic and dopaminergic receptors[3-6]. We were interested in comparing d-LSD binding (capacity and affinity) in serotoninergic and dopaminergic nuclei with that in some of their projection areas.

*Experimental.* Adult male albino Wistar rats (200–250 g) were kept under controlled conditions of light:dark (12 h:12 h), temperature 24 °C and feeding ad libitum. Tritium-labelled d-LSD (Amersham 15.3 Ci/mmole; 1 µg/kg in 100 µl 0.1% ascorbic acid) was injected i.v. via the tail vein. In displacement experiments increasing doses of cold d-LSD were simultaneously injected i.v. with $^3$H-d-LSD. The rats were decapitated 30 min after the injection and their brains rapidly removed and frozen on dry ice. Transverse parallel slices (1 mm) were prepared on a slicing apparatus at a temperature of −20 °C. Different regions and nuclei were located using König and Kippel's atlas[7] and were punched out with a cooled metallic needle (diameter 1.5 mm). Each region was solubilizied with 0.2 ml Soluene (Packard), acidified with $H_2SO_4$ and counted for 20 min (Instagel, Packard) in a tricarb scintillation counter. In the cerebellum only a negligible amount of the

$^3$H-dLSD binding in brain regions expressed as the ratio of the respective brain part to cerebellum (1 µg/kg $^3$H-d-LSD)

| | Mean values ± SEM | No. of rats |
|---|---|---|
| Frontal cortex | 3.03 ± 0.24 | 11 |
| Striatum | 2.65 ± 0.23 | 5 |
| Parietal cortex | 2.36 ± 0.09 | 11 |
| Hippocampus | 1.83 ± 0.09 | 5 |
| Occipital cortex | 1.81 ± 0.09 | 11 |
| Substantia nigra | 1.72 ± 0.07 | 11 |
| Raphe nuclei d + m | 1.53 ± 0.07 | 11 |
| Cerebellum | 1.00 | |

d-LSD binding was saturable and no regional differences were found. This allowed us to express d-LSD binding as the ratio of the amount found in the respective brain region to that found in the cerebellum (the cerebellum being used as an internal standard).

*Results and discussion.* In the cortex specific $^3$H-d-LSD binding increased steadily from the occipital to the frontal cortex whereas non-specific binding (co-injection of 1 mg/kg cold LSD) changed only slightly (figure 1). In the striatum, cerebellum, hippocampus, substantia nigra and raphe nuclei no concentration gradient was detectable. Our results confirm the in vitro binding studies with monkey cortex which also showed the highest binding in the frontal part[8]. As was found for d-LSD, $^3$H-spiroperidol in the rat cortex, in vitro and in vivo, binds preferentially to the frontal part[9], where it occupies one of the 2 postulated serotonin receptors which are both binding sites for d-LSD[10].

As can be seen from the table, the substantia nigra and raphe nuclei bind d-LSD to a lesser extent than cortex, striatum and hippocampus. The affinity of d-LSD for its binding-sites was determined by displacement experiments in each region (figure 2). The most remarkable result is an $IC_{50}$ value in the substantia nigra about 10 times higher than that found in the other regions. The binding sites in the substantia nigra therefore have a lower affinity for d-LSD. The striatum also has different binding characteristics, but in contrast to the substantia nigra it has a lower $IC_{50}$ value than the 3 brain parts with identical binding characteristics (cortex, hippocampus, raphe nuclei). Moreover, in figure 2 the slope for the striatum is markedly different. This finding may reflect the participation of different types of receptors in striatal d-LSD binding (e.g. serotonin and dopamine). 2 types of DA-receptors are known, adenylcyclase linked ($D_1$) and unassociated ($D_2$)[11]. d-LSD can cause accumulation of c-AMP in the striatum but not in the substantia nigra[12]. Furthermore d-LSD significantly decreases plasma prolactin in rats by stimulation of dopamine receptors in the pituitary[13] a gland without dopaminesensitive adenylcyclase. These findings indirectly suggest that d-LSD binds in the substantia nigra, in contrast to the striatum, mainly to $D_2$-dopamine receptors; this could explain the observed differences in the $IC_{50}$ values.

Haigler and Aghajanian concluded from their electrophysiological investigations that d-LSD is more efficacious on presynaptic serotonin receptors in the raphe nuclei than on postsynaptic ones (e.g. in the septum)[4]. In contrast, the raphe nuclei do not show an exceptionally high amount of specific binding of d-LSD nor have these binding sites a special affinity, as is shown by a similar $IC_{50}$ value (5 µg/kg) in the serotoninergic regions e.g., the raphe nuclei, hippocampus, striatum and cortex. In other words, the electrophysiological effect of d-LSD does not depend solely on the formation of receptor-d-LSD complex. Different authors[4,14] report an $ED_{50}$ value of inhibition of raphe firing comparable to the $IC_{50}$ value found in our displacement experiments (figure 2). Thus the firing activity of these nuclei seems to correspond linearly with d-LSD binding.

Our method allows specific d-LSD binding to be determined in small brain regions. The method may prove useful as an additional means of describing receptors, completing other in vivo approaches such as electrophysiological ones.

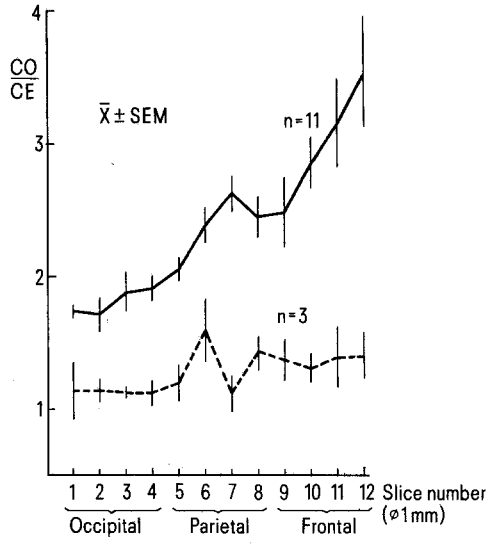


Fig. 1. Regional distribution of $^3$H-d-LSD binding in cortex expressed as the ratio of cortex to cerebellum. ——, 1 µg/kg $^3$H-d-LSD alone; – – –, co-injection of 1 mg/kg cold d-LSD.

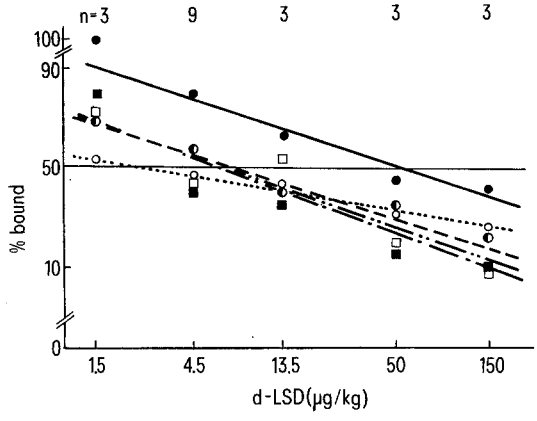


Fig. 2. Displacement of $^3$H-d-LSD from the brain regions by unlabelled d-LSD (log-probit-analysis). 100%: 1 µg/kg $^3$H-d-LSD alone; 0%: co-injection of 1 mg/kg cold d-LSD. ●, Substantia nigra; ○, striatum; ■, raphe nuclei d+m; ◐, cortex all parts; □, hippocampus.

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