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Rapid communication

SEROTONIN RECEPTOR LOCALIZATION IN RAT BRAIN BY LIGHT MICROSCOPIC AUTORADIOGRAPHY

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Both LSD and 5-HT are useful for labeling serotonin receptors in binding assays (Fanow and Van Vunakis, 1973; Bennet and Snyder, 1976). In this preliminary report, we have utilized an in vitro labeling, light microscopic autoradiographic technique (Young and Kuhar, 1979) to investigate the distribution of LSD binding and serotonin binding receptors in several areas of the rat brain. The procedure is as follows. Sections (6 μ) of rat brain were cut on a cryostat microtome and thaw-mounted onto subbed slides. 5-HT receptors were labeled by incubation of the slide-mounted sections in 1.0 nM ^{3}H-5-HT (New England Nuclear, Boston, MA; 30 Ci/mmole) for 60 min at 20°C in 0.17 M Tris-HCl buffer, pH 7.6 with 0.001% ascorbic acid, 10 μM pargyline and 4 mM $CaCl_2$. ^{3}H-LSD (Amersham, Arlington Heights, IL; 3.1 nM, 38 Ci/mmole) was incubated under identical conditions. Blanks were generated by adding 1 μM LSD for both ligands during a 5 min preincubation as well as the incubation. Additional sections incubated with ^{3}H-LSD also contained 100 nM lisuride and/or 100 μM mescaline. Following two consecutive 5 min washes in cold buffer without drug, the mounted tissue sections were dried and processed for autoradiography (exposure duration: 9 weeks) as previously described (Young and Kuhar, 1979). Preliminary biochemical experiments with sections showed that these incubation conditions gave an optimal labeling of receptors and provided specific to non-specific ratios of 5-10 to 1 for ^{3}H-LSD and 5-HT. The binding, which was unaffected by our preparative procedure, exhibited saturability, high affinity and appropriate kinetics. In addition, various drugs exhibited proper potencies in displacing the tritiated ligands (Details in preparation).

The 5-HT receptors displayed heterogeneous distributions throughout the CNS. ^{3}H-5-HT binding sites were particularly high in the dentate gyrus (molecular layer), CA3 and CA4 (not in hilus and not over pyramidal cells) of the hippocampal formation, nucleus parolivaris superior, superior colliculus, median raphe and spinal substantia gelatinosa. Very high levels were found in the choroid plexus, subiculum and dorsal raphe (fig. 1). Moderate levels were seen in CA1 and CA2 of the hippocampus, the substantia nigra pars compacta, inferior colliculus and Rexed area 10 of the spinal cord. ^{3}H-LSD binding paralleled the ^{3}H-5HT binding in all areas and was reduced to background levels in the presence of 100 nM lisuride whereas mescaline (100 μM) produced only uniform partial reductions.

Recently, it has been postulated that there are two distinct 5-HT receptors, one labeled by ^{3}H-5-HT, the other labeled by ^{3}H-spiperone; ^{3}H-LSD labels both (Peroutka and

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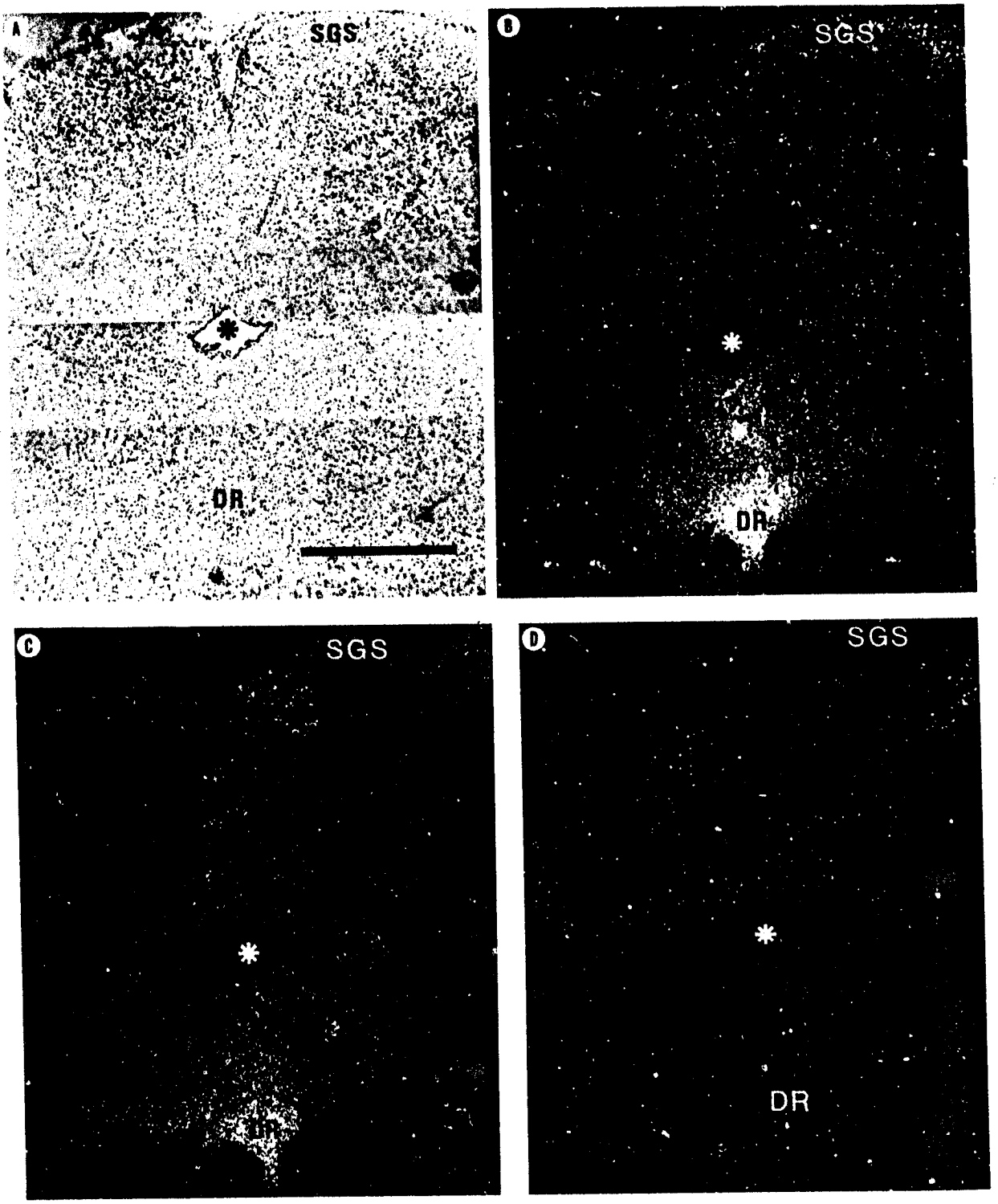

Fig. 1. Autoradiographic distribution of 5-HT receptors in rat midbrain. Brightfield micrograph in A shows tissue with superior colliculus (SGS, stratum griseum superficiale), dorsal raphe nucleus (DR) and the cerebral aqueduct (*). Darkfield micrograph showing autoradiographic grains in B (same slide as A) demonstrates ^{3}H-LSD binding sites while micrograph in C (from an adjacent tissue section) shows ^{3}H-5-HT binding sites. High concentrations of receptors are found in the dorsal raphe nucleus with lower densities in the periaqueductal grey and superior colliculus. The darkfield micrograph in D is a blank produced by adding 1 μM LSD to ^{3}H-5HT-containing incubation media (see text). These results were highly reproducible, found in several sections from the same and different animals. Bar = 1 mm.

Snyder, 1979). In preliminary experiments, we were able to detect some LSD displaceable ^{3}H-spiperone binding in the cortex and facial nerve nucleus suggesting the presence of the second type of receptor in those areas. We also observed in several areas (e.g., pyramidal cell layer, and, to lesser extent, its dendritic fields in CA1 of the hippocampus) ^{3}H-spiperone binding which was not displaced by sulpiride, LSD or norepinephrine (in preparation).

Our observed distributions of 5-HT receptors are compatible with several histochemical and electrophysiological studies in other laboratories (Haigler and Aghajanian, 1977). These radiohistochemical studies should point out target sites for further study of these receptors and should be a valuable addition to other histochemical methods for mapping functional serotonergic pathways.

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