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Rapid communication

QUANTITATIVE AUTORADIOGRAPHY OF NEUROTRANSMITTER RECEPTORS USING TRITIUM-SENSITIVE FILM *

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In the past 3 years techniques have been developed for autoradiographic localization of diffusable compounds including neurotransmitter receptor ligands (Young and Kuhar, 1979; Herkenham and Pert, 1980). The high spatial resolution of these techniques has provided elegant demonstrations of the regional heterogeneity of receptor densities within the CNS, however, this is achieved at the expense of the direct quantitation capabilities of homogenate binding methods. We have, therefore, developed an autoradiographic technique using tritium sensitive film (LKB Ultrofilm ^{3}H) to achieve rapid high resolution quantification of benzodiazepine, GABA and glycine receptor numbers and affinities in multiple areas of the central nervous system.

For the receptor assays, frozen sections of brain and spinal cord were mounted on gelatin coated slides at room temperature and stored at −20°C. Prior to assay, the slides were given three consecutive 5 min washes in 50 mM Tris-citrate buffer (pH 7.2) at 0°C. They were then incubated for 30 min at 0°C in solutions of either [^{3}H]flunitrazepam (1-15 nM, 88 Ci/mmol) or [^{3}H]muscimol (5-80 nM, 19 Ci/mmol) in 50 mM Tris citrate buffer pH 7.2, or [^{3}H]strychnine (5-80 nM, 15 Ci/mmol) in 50 mM Na-K phosphate buffer (pH 7.0). Adjacent sections were incubated with excess diazepam (2×10^{-6} M), GABA (10^{-3} M) or glycine (10^{-3} M) to determine nonspecific binding. Sections were given 3 quick rinses in cold buffer, blown dry with cold air, taken to a dark room, placed in an X-ray cassette along with carbon-14 standards, apposed to a sheet of Ultrofilm ^{3}H and exposed in the dark at 4°C for 3-12 days. The film was then removed from the sections, developed in D_{19} for 5 min at 20°C, fixed, washed and dried. Typical autoradiograms are shown in fig. 1. The film was then placed in a photographic enlarger and the optical densities of areas of film were determined with a photodiode densitometer.

For any given area, 8 independent readings were made, averaged and compared to standards to determine the femtomoles of ligand bound per mg tissue. From each point was subtracted the densitometric reading (average of 8 readings) of comparable regions on the blanks slides. Data from the film, obtained from tissue sections dipped in several different concentrations of ligand, were plotted according to Scatchard. These plots yielded straight lines with little variation of K_Ds among regions (K_Ds were 28 ± 4 nM for [^{3}H]muscimol, 14 ± 1 nM for [^{3}H]strychnine and 4 ± 0.4 nM for [^{3}H]flunitrazepam). Among different areas, B_{max} varied widely (fig. 1). The values are compatible with data from homogenate studies as well as with those from receptor autoradiography (Young and Kuhar, 1980; Zarbin et al., 1981).

The use of tritium sensitive film has several advantages over the previously described techniques for receptor autoradiography using emulsions (Young and Kuhar, 1979; Herkenham and Pert, 1980). The technique is simple, the exposure

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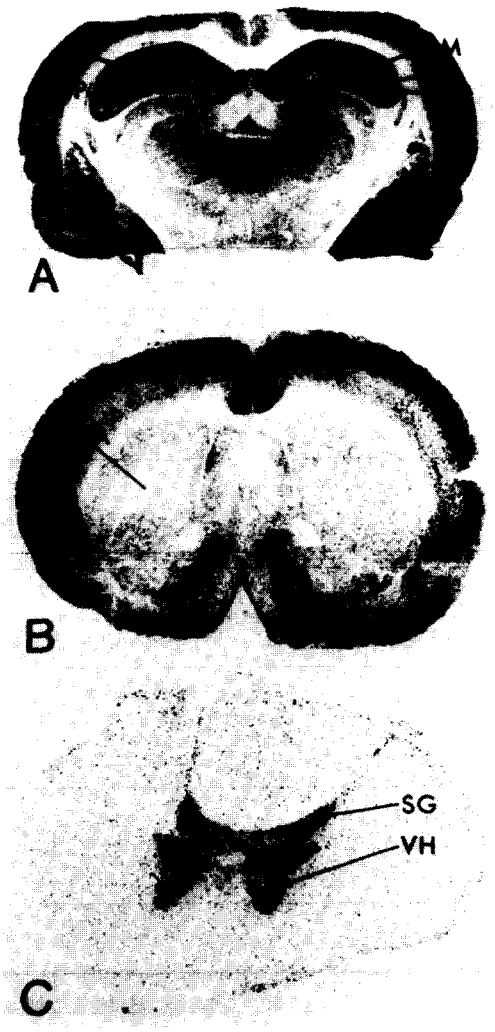

Fig. 1. Representative receptor ligand autoradiograms. (A) [^{3}H]Flunitrazepam binding in rat forebrain. Binding constants and receptor densities were determined autoradiographically in respective areas: Stratum oriens (SO) (B_{max} = 0.44 pmol/mg tissue, K_D = 5.1 nM); stratum radiatum (SR) (B_{max} = 0.40 pmol/mg tissue, K_D = 5.5 nM); stratum moleculare (SM) (B_{max} = 0.53 pmol/mg tissue, K_D = 4.5 nM); stratum pyramidale (SP) of hippocampus (B_{max} = 0.20 pmol/mg tissue, K_D = 3.7 nM) and the hilus of the dentate gyrus (DH) (B_{max} = 0.20 pmol/mg tissue, K_D = 3.8nM). (B) [^{3}H]Muscimol binding in rat forebrain. Note decreased binding over the left striatum following local injection of kainic acid 60 days before sacrifice (KA), suggesting that GABA receptors are localized on intrinsic striatal neurons. (C) [^{3}H]Strychnine binding in monkey thoracic spinal cord. Highest receptor density is in substantia gelatinosa (SG) (B_{max} = 2.1 pmol/mg protein, K_D = 23 nM), while the ventral horn (VH) contains fewer receptor sites of similar affinity (B_{max} = 1.6 pmol/mg protein, K_D = 16 nM).

time is shorter than with previous techniques, and the film can be removed from the section to perform densitometry. It is the latter property which imparts power to the method since it permits application of quantitative autoradiographic techniques (Sokoloff et al., 1977) to receptor pharmacology. The technique allows the regional determination of receptor numbers, affinities, binding kinetics, and competitive inhibition constants in brain with spatial resolution as fine as 100 μm^2.

Thus, receptor pharmacology in areas too small to study easily with conventional techniques (e.g. entopeduncular nucleus) as well as regional differences within larger areas (e.g. hippocampus) can be approached quantitatively. The method may prove invaluable in examining the regional distributions of receptor subtypes, or in rapidly determining the responses of receptors in multiple areas to brain lesions or drug treatments. The principles underlying the current method may be further extended to other receptor-ligand systems studied with previous autoradiographic techniques.

References

Herkenham, M. and C.B. Pert, 1980, In vitro autoradiography of opiate receptors in rat brain suggests loci of 'opiatergic' pathways, Proc. Natl. Acad. Sci. U.S.A. 77, 5532.

Sokoloff, L., N. Reivich, C. Kennedy, M.H. Des Rosiers, C.S. Patlack, K.D. Pettigrew, O. Sakurada and M. Shinohara, 1977, The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure and normal values in the conscious and anesthetized albino rat, J. Neurochem. 28, 897.

Young, W.S. and M.J. Kuhar, 1979, New method for autoradiography: [^{3}H]opioid receptors in rat brain, Brain Res. 179, 255.

Young, W.S. and M.J. Kuhar, 1980, Radiohistochemical localization of benzodiazepine receptors in rat brain, J. Pharmacol. Exp. Ther. 212, 337.

Zarbin, M.A., J.K. Wamsley and M.J. Kuhar, Glycine receptor: light microscopic autoradiographic localization with ^{3}H-sfrychnine, J. Neurosci. (in press).