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COMPUTER ASSISTED IMAGE ANALYSIS OF TRANSMITTER RECEPTORS

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ABSTRACT

Quantitative methodologies for the analysis of autoradiograms have been developed using computerized image analyzers coupled to standard TV camera input for microdensitometrical evaluations. The procedures include the assessment of the film response to radioactivity using appropriate standards calibrated according to brain tissue quenching (grey matter) and a non-linear conversion of optical density measurements into radioactivity values. In this procedure the best fitting mathematical model has been adopted to give as low variability as possible in the transformation of the data. The reliability of this approach has been evaluated both by means of computer assisted Monte-Carlo simulation procedures and by parallell biochemical determinations of the binding characteristics of transmitter receptors.

Key-words: Brain tissue quenching, film response, Monte-Carlo simulation procedures, receptor autoradiography.

INTRODUCTION

A general trend in the last years of neuroscience research has been the development of reliable quantitative methods in neuroanatomy to study transmitter identified neuronal systems in the brain. In this way, quantitation available with biochemical methods can be applied to the higher resolution level allowed by histochemistry. This is particularly important in the study of the central nervous system, where structure-function relationships are very relevant. During recent years receptor autoradiography has been extensively used to study the distribution of neurotransmitter receptors in the brain (Kuhar 1981) and also quantitative microdensitometrical methods have been developed to study receptor parameters and their changes after different experimental conditions (Palacios et al., 1981; Unnerstall et al., 1981, 1982; Rainbow et al., 1982; Fuxe et al., 1983; Agnati et al., 1984, Benfenati et al., 1985). Because of the always wider availability of computerized image analysers, autoradiography is carried out in two dimensions using these devices. However, the most common input of these systems is represented by a standard scanning TV camera which cannot be actually considered a microdensitometrical system,

having a fast scanning speed and a low signal/noise ratio. To overcome this disadvantage image enhancement and averaging or an expensive low scanning solid state camera may be used. In the present paper the procedure for the quantitation of receptor autoradiograms will be presented with special reference to tissue quenching, emulsion response to radioactivity, density data conversion and final evaluation of binding results. The reliability of the densitometrical evaluation performed using a standard TV camera input has also been assessed and the best data conversion models defined in order to minimize the effects of random density reading errors on the final binding data.

METHODOLOGICAL ASPECTS

Image analyzer

The density values of the autoradiographic areas have been measured using the digital image analyzer Tesak VDC 501 coupled to a PDP 11/23 computer. This apparatus resolves the image in 512x512 pixels and codifies density values in a digital scale ranging from 0 (full absorption) to 255 (full transmittance). After the measure of the sample area (A) and of the respective background (BK), we chose to express the final density evaluation as follows:

Absorption (%)= 100 - (A*100/BK)

that corresponds to 100% minus the per cent ratio between sampled area and background transmittance. This value seems to be the most reliable optical density parameter, and it has been used throughout this analysis.

Evaluation of tissue quenching and of the emulsion response to radioactivity

The direct relationship between the optical density of the autoradiograms of the brain sections and the true amount of labeled ligand bound to the tissue is affected by tissue quenching (particularly relevant if β-particles are used as tracers) and by the non linear emulsion response to radioactivity. To correct for these factors it is necessary to analyze the emulsion response to increasing standard amounts of radioactivity quenched by the tissue and to different exposure times (Fig. 1.). This dose-response relationship has been fitted using different mathematical models, namely a 2 parameter hyperbolic function and a 4 parameter logistic function. The actual radioactivity bound to an unknown labeled area (dpm/mm^2) can then be obtained by simply interpolating its absorption value in the fitted curve. The values can then be transformed into fmoles/mm^2 by knowing the specific activity of the radioligand. This value was subsequently transformed into fmoles/mg prot by measuring the protein contents of the 14 μm thick brain sections, used in the tissue standards (see Fig. 2), with reference to their area expressed in mm^2. 1 fmol/mm^2 was found to correspond to 510 fmoles/mg protein. By plotting on the x-axis the logarithm of the total energy (in days dpm/mm^2) it is possible to merge absorption data obtained from differently exposed standard curves and to fit a "cumulative characteristic curve". This curve is useful when predicting the right exposure time and for density - radioactivity conversions if the biological samples have an exposure time different from that of the standard curve. As seen in Fig. 2 both standards show a high quenching activity (similar ED50 values) but the tissue standard gives a less steep curve and thus a very wide range of activity. In this way excellent dose-response curves can be obtained over a wide range of exposure times. The section area used to induce quenching was rich in gray matter (e.g. striatum). The section was applied onto dried drops (2 μl) of

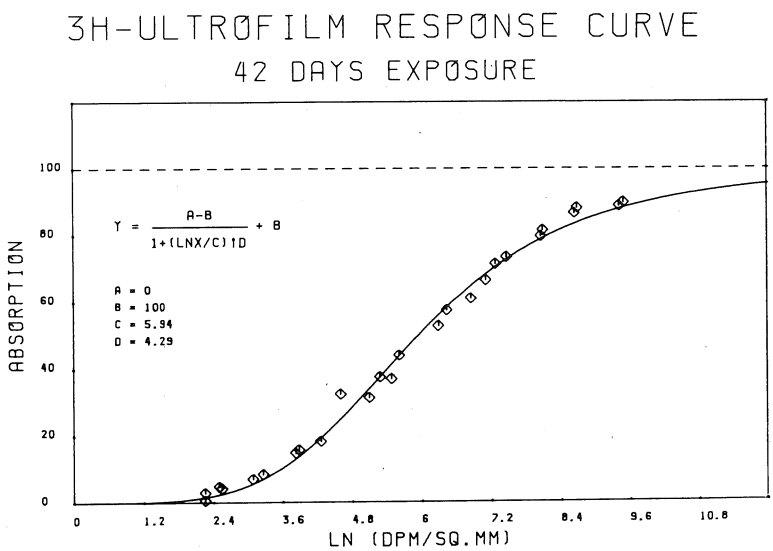

Fig. 1. Emulsion response to increasing energy amounts fitted using a 4 parameter logistic function.

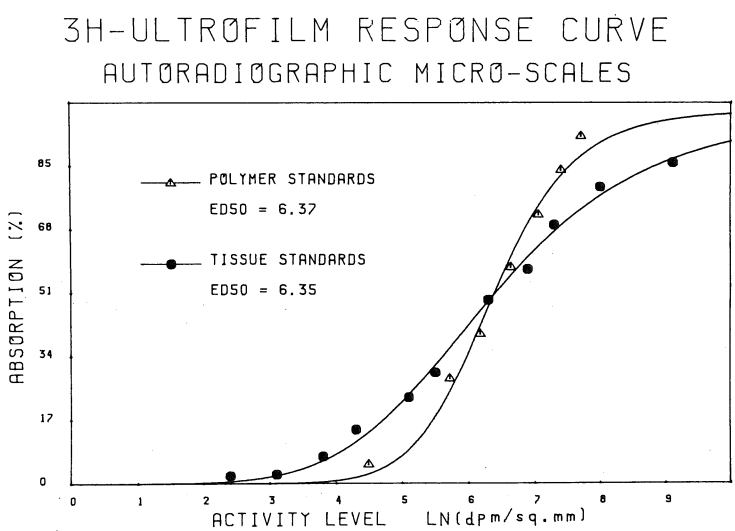

Fig. 2. Studies on the effects of polymer standards (^{3}H-microscales, Amersham, activity range 3-110 nCi/mg) and tissue standards (gray matter in sections) on the optical density-activity level relationship.

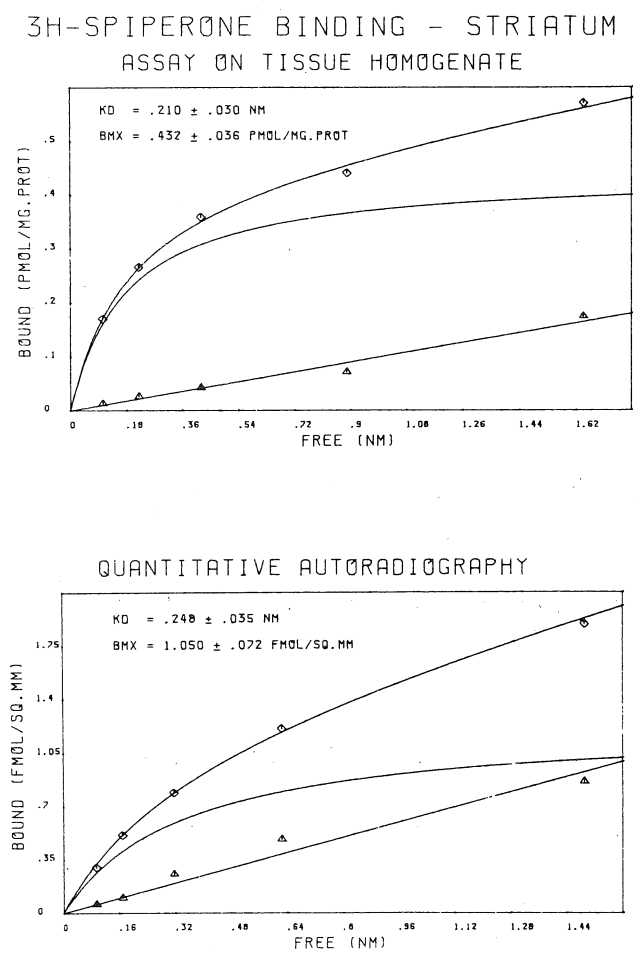

Fig. 3. Studies on the binding characteristics of ^{3}H-spiperone in striatum of rat using biochemical procedures (tissue homogenates) or quantitative receptor autoradiography. The K_D and B_{max} values obtained are very similar. The procedure used in that of Creese et al., 1977. Non-linear fitting procedures.

standard melted and kept in a wet chamber to allow complete isotope diffusion. The selection of adequate quenching conditions has been found to be important to obtain reliable basal values after conversion of the optical density values. Using our tissue standard we have had excellent agreement between results obtained in biochemical and quantitative receptor autoradiographical studies e.g. on the binding characteristics of DA D2 receptors in striatum (^{3}H-spiperone) (see Fig. 3).

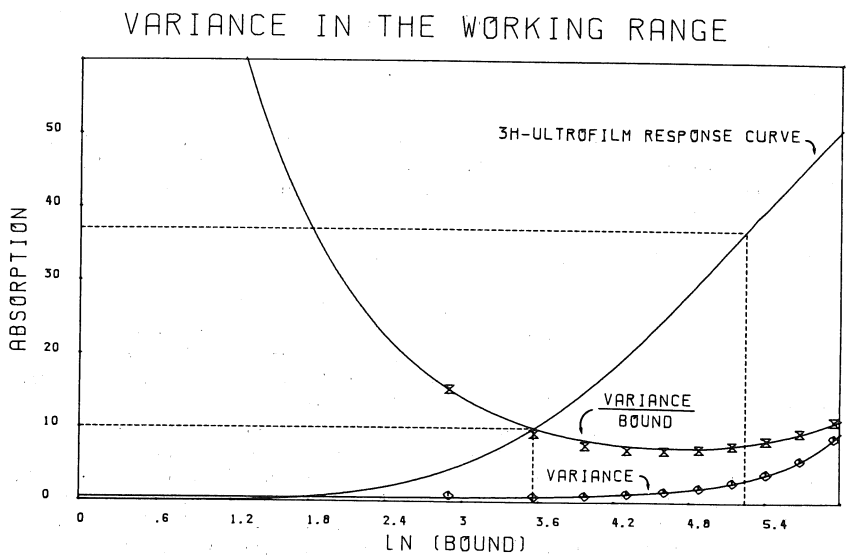

Fig. 4. Changes of variance and per cent variance for different replications simulated with random errors and performed at various absorption levels.

STATISTICAL ANALYSIS

The interpolation with non linear functions introduces a potential error in the transformation of normally distributed observations. In order to assess this phenomenon, we performed a Montecarlo simulation by introducing, for various absorption levels, random errors in density measurements and analyzing variance in the transformed data. From these studies it was apparent that variance and per cent variance are very low in an absorption range 10-40% (working range), whereas the calculated bound levels are less reliable, when different optical density levels are considered. Furthermore, the logistic model was found to be optimal, giving lower degrees of variability in the final determinations (Fig. 4). In this way also a mathematical model of variance pattern becomes available and it becomes possible to introduce weights to offset the tendancy for unreliable points to influence the location of the curve and the final parameter determination.

BIOLOGICAL ASPECTS

Quantitative studies of receptors in various experimental conditions

By using this methodological approach we have determined the biochemical parameters of transmitter receptors in discrete brain areas, obtaining results in close agreement with biochemical methods. We have also studied area-specific receptor responses to different experimental conditions such as ion concentrations, in vivo injection of irreversible ligands, chronic receptor blockade, presence of neuropeptides or age-induced changes. Some of these results will be presented in order to show the potentialities and the high resolution level of receptor autoradio-

graphy to detect subtle morphofunctional changes in transmitter-identified neuronal systems.

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