Radioimagers as an alternative to film autoradiography for \textit{in situ} quantitative analysis of $^{125}$I-ligand receptor binding and pharmacological studies

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Summary

Three radioimagers, the $\mu$-imager, the $\beta$-imager and the phosphorimager, were tested as alternatives to quantitative autoradiography on film, for receptor imaging and pharmacological \textit{in situ} quantitative analysis. Two iodinated ligands $^{125}$I-interleukin-1$\alpha$ and $^{125}$I-gonadotropin releasing hormone agonist, were used for receptor characterization in mouse brain and pituitary sections. Due to the high number of the agonist receptors in rat pituitary gland, this tissue was used to compare measurements obtained from digital autoradiograms with classical $\gamma$ detector determination. This permits the evaluation of radioimager efficiency and absolute quantification.

Radioimagers represent an improvement in terms of time of image acquisition. All the radioimagers are more sensitive than film for the detection of low levels of radioactivity. The spatial resolution provided by the $\mu$-imager compares favourably with that obtained on film autoradiograms while digital autoradiograms from the phosphorimager and $\beta$-imager did not show precise definition under our experimental conditions. Superimposition of histological structures from the stained sections with radiolabelled areas in the autoradiograms remains, at this time, the unique advantage of film.

In conclusion, radioimagers represent an alternative to autoradiography on film or emulsion for \textit{in situ} quantitative studies on tissue sections. They combine precise imaging for \textit{in situ} binding studies with easy and direct access to counts in cpm. The improvement in radioimaging technology has, therefore, brought \textit{in situ} analysis of iodinated ligand binding to the level of accuracy that is obtained with classical detectors of radioactivity.

Introduction

Pharmacological studies on receptors for various peptides, steroids or other ligands were first carried out on tissue homogenates, membrane preparations or isolated cells. These techniques have proved very useful for receptor characterization but had to be complemented by autoradiographic studies on slide-mounted tissue sections for receptor localization.

During the last decade, autoradiograms on film or micro-autoradiograms on histological sections have been quantified using image analysers calibrated with known standards (Kuhar \textit{et al.}, 1991). The determination of receptor specificity and affinity became possible on very small structures of interest using quantitative autoradiography (Palacios \textit{et al.}, 1981; Rostène \textit{et al.}, 1984; Leblanc \textit{et al.}, 1988; Haour \textit{et al.}, 1990; Ban \textit{et al.}, 1991; Cruméyrolle-Arias \textit{et al.}, 1993; Vincens \textit{et al.}, 1993; Jafarian-Tehrani \textit{et al.}, 1994; Marquette \textit{et al.}, 1995). The technique was complicated, however, both by the restricted scale of linearity of the response of the photographic emulsion over a wide range of radioactive concentrations (Kuhar \& Unnerstall, 1985, 1990) and by the length of exposure time. In many studies therefore, the autoradiographic studies remained comparative or qualitative.

New high-resolution radioimagers developed recently could represent alternatives to autoradiography on film (Cruméyrolle-Arias \textit{et al.}, 1994) and micro-autoradiography on tissue sections (Lanièce \textit{et al.}, 1994, 1996) for \textit{in situ} quantitative studies on tissue sections.
They are rapid and sensitive with a wide dose-response linearity (Charpak et al., 1989; Theler et al., 1993; Lanièce et al., 1994).

This paper will illustrate and analyse binding experiments obtained with 3 different radioimagers using iodinated radioligands (a neuro-hormone, gonadotropin releasing hormone, and a cytokine, interleukin-l) on rodent brain and pituitary tissue sections. The aim of the present study was to test the capacity of radioimagers to combine the advantages of in situ studies on tissue sections (i.e. precision of localization and sensitivity) with those of classical receptor studies on tissue preparations (i.e. speed and absolute quantification).

Materials and methods

TISSUES

Brains and pituitaries from 3 month-old male Wistar rats and C3H/He/OU mice (C. Rivers Breeding, St Aubin les Elbeuf, France, and Iffa-Credo, St Germain S/l'Abresle, France, respectively) were obtained after sacrifice by decapitation. Organs were frozen in dry ice and stored at -70°C until sectioning. Tissue sections were 20 μm thick (~18°C, Frigocut 2800, Reichert-Jung), and were mounted on chrome alum gelatin-coated slides and stored at -20°C until used for the binding experiments.

HORMONES

Gonadotropin hormone releasing agonist (GnRHa: [D-Ala6, Des-Gly10]-GnRH ethylamide, Peninsula, England) was labelled with 125I by the chloramine T method (Nett et al., 1973) as described previously (Leblanc et al., 1988). 125I recombinant human interleukin-1α (rhIL-1α) was obtained from Amer sham (les Ulis, France). The specific activities of iodinated ligands were 1500 and 2000-2500 Ci.mmol⁻¹ for 125I-GnRHa and 125I-rhIL-1α respectively. Unlabelled hormones were GnRHa from Peninsula (England) and rhIL-1α from Dr S. Gillis (Immunex Corporation, Seattle, USA).

IN VITRO BINDING ON TISSUE SECTIONS

Binding experiments were done on serial sections through the hippocampus and the pituitary. Incubation of tissue sections was carried out as previously described (Crume yrolle-Arias et al., 1994; Jafarian-Tehrani et al., 1994) using 0.08 to 8.3 nmol.L⁻¹ 125I-GnRHa and 0.20 or 0.58 nmol.L⁻¹ 125I-rhIL-1α (see legends of figures for precise concentrations). Incubations were performed at 4°C (1 h) or 20°C (2 h) for 125I-GnRHa and 125I-rhIL-1α respectively. The amount of non-specific labelling was evaluated on adjacent sections in the presence of an excess of non-radioactive ligand (GnRHa: 1 μM and rhIL-1α: 0.1 μM).

RADIOIMAGERS

Phosphorimager

The sensitive surface is a plate composed of fine crystals of BaFBr:Eu²⁺ in an organic binder (Amemiya & Miyahara, 1988). This reusable phosphor screen stores the pattern of radioactivity by crystal excitation after close contact with the radioactive source, and reconstructs the latent image by emission of luminescence at 365 nm by scanning with a helium-neon laser at 635 nm.

β-Imager

The ionised particles emitted by the sample initialize an avalanche of secondary particles in a gas chamber, under the influence of electric fields between parallel electrodes. The gas mixture was triethylamine added to argon. The ionized particles induce the emission of spots of light that are intensified and finally detected by a CCD camera (Charpak et al., 1989).

µ-Imager

The sensitive surface is a thin scintillator sheet (10 μm thick. Y₂SiO₅(Ce)) coupled with an intensified CCD (charged coupled device) system driven in a specific mode (Charon et al., 1991). Its basic principle is to convert the detected particle energy locally into light and secondarily, to intensify the corresponding light spot to display the emission point localized by the CCD on a PC screen (Lanièce et al., 1994).

GENERATION AND ANALYSIS OF AUTORAD1OGRAMS

The use of radioimagers requires dry, labelled tissue sections similar to those used for autoradiography on film. Radioimagers generate digital autoradiograms, in contrast to silver grain autoradiograms obtained after the standard procedure of development of photographic emulsion laid upon films or tissue sections. Quantification of optical densities of autoradiograms on film (³H-Hyperfilm, Amersham, Les Ulis, France) was obtained using the Biocom RAG 200 image analyser (Biocom, Les Ulis, France) and converted into radioactive units by comparison with iodinated standards (Amersham microscale, batch 4). Radioimagers allow the direct measurement of the level of radio-activity on digital autoradiograms (counts per mm²). For absolute quantification (dpm per mm²), each radioimager must be calibrated with standards for the corresponding isotope.

DATA ANALYSIS

Results of quantifications were analysed by non-linear regression (GraphPad Prism, version 1.0, GraphPad Software, San Diego, USA). The dissociation constant (Kd) and the maximal binding (Bmax) were calculated from a saturation experiment performed on serial sections.

Results

IN SITU QUANTITATIVE ANALYSIS USING AUTORAD1OGRAPHY ON FILM

Using serial tissue sections, the affinity and specificity of ligand-receptor interactions can be studied using autoradiographic images obtained on film after in vitro binding experiments. The quality and resolution of the images allow receptor characterization in very small