Autoradiographical and immunohistochemical analysis of receptor localization in the central nervous system

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Summary
Quantitative receptor autoradiographic methods have been widely used over the past two decades. Some of the advantages and limitations of these techniques are reviewed here. Comparison with immunohistochemical and in situ hybridization methods is also highlighted, as well as the use of these approaches to study receptor gene over-expression in cell lines. Together, data obtained using these various methodologies can provide unique information on the potential physiological roles of a given receptor protein and/or binding sites in various tissues.

Introduction
Over the past three decades, the use of radioligand membrane binding techniques has permitted the detailed pharmacological characterization of receptor sites in both peripheral tissues and in the brain (Yamamura et al., 1978; Snyder, 1983; Quirion & Gaudreau, 1985; Keen & MacDermot, 1993). In addition, the development of selective agonists and/or antagonists has provided the opportunity to distinguish different subtypes in a given receptor family (Lee et al., 1986; Goldstein & Naidu, 1989; Dennis et al., 1990; Dumont et al., 1995). However, one of the major limitations of this technique is the lack of resolution at the anatomical level. The differential distribution of binding sites in heterogeneous tissues like the central nervous system (CNS) is practically impossible to obtain with membrane binding studies. In order to overcome this problem, receptor autoradiography has been developed. This anatomical technique has been the method of choice for studying the differential distribution of a great number of receptors. While this method permits an excellent macroscopic localization of binding sites, its resolution at the cellular level using photographic emulsion is rather poor, and it is difficult to combine it with other methods that require tissue fixation. These inherent limitations can, however, be overcome with the use of in situ hybridization histochemistry and immunohistochemistry. The former approach is based on the site of localization of receptor messenger RNA, thereby visualizing the cell bodies that synthesize a given receptor. Immunohistochemical techniques, on the other hand, provide cellular localization of receptor proteins using specific monoclonal or polyclonal antibodies. A better understanding of the anatomical organization of a given receptor class can therefore be achieved by the overall analysis of data obtained using these three complementary approaches. In this article, we focus primarily on the multiple applications of these various approaches in the understanding of the physiological relevance of a given receptor. Readers are directed to a very recent article for practical guidelines on in vitro receptor autoradiography and in situ hybridization, as this topic will not be discussed in this article (Sharif, 1996).

Receptor autoradiography
Receptor autoradiography is a method that takes advantage of the ability of a given receptor binding site, present in a specific tissue, to bind a radiolabelled ligand, and of the feasibility of localizing the bound
probe by several autoradiographic techniques. The accuracy of the resulting autoradiogram depends on many factors, including the type of radioligand used (irreversible or reversible ligands), the autoradiographic method (in vivo or in vitro labelling) and the photographic medium (wet photographic emulsion or dry emulsion-coated coverslips or radiosensitive films) employed to record the radioactive emissions. One of the most important points to be considered is the avoidance of any diffusion of the radiolabelled molecules bound to the receptor of interest, until the autoradiogram is formed. One way to overcome this problem is to use irreversible radioligands (Kuhar & Yamamura, 1975, 1976; Rotter et al., 1979; Kuhar et al., 1981). Since this type of probe is covalently bound, various photographic means can be selected for the detection of the bound radioactivity. However, one major disadvantage of using irreversible ligands often relates to relatively high levels of non-specific binding.

Accordingly, the majority of autoradiographic methods involve the use of reversible ligands, which can readily dissociate from their binding sites in an aqueous environment. In this case, the wet photographic emulsion technique is limited, as a liquid emulsion is applied directly on to unfixed tissue sections. To overcome this problem, two approaches, depending on the molecular nature of the ligand, have been used. A radioligand possessing a free amino group not involved in ligand-receptor interactions may be covalently linked to the receptor by the use of a chemical fixation method (for example, aldehyde fixing agents such as paraformaldehyde or glutaraldehyde) before the application of the wet photographic emulsion (Herkenham & Pert, 1982). Alternatively, to avoid the diffusion of the radioligand, Young and Kuhar (1979) have applied the dry apposition technique of Stumpf and Roth (1966) to receptor autoradiography. This involves the application of a liquid photographic emulsion on to a glass coverslip, followed by the apposition of the dried emulsion-coated coverslip to the labelled tissue section.

Subsequently, the development of commercial emulsion-coated plastic films has allowed the widespread use of the dry application method for the visualization of diffusible radioactive ligands (Palacios et al., 1981; Quirion et al., 1981; Unnerstall et al., 1982). These radiosensitive films provide a reasonable degree of resolution, permitting the macroscopic localization of binding sites, and the possibility of quantifying the apparent density of receptors with the help of computer-assisted image analysis systems.

The choice of detection methods to visualize radiolabelled ligand/binding site complexes is determined by the degree of resolution required. For gross anatomical resolution, autoradiograms produced using emulsion-coated films or digital images derived from phosphor plate imagers (Appel et al., 1991) should be employed. The dry emulsion-coated coverslip technique or the direct dipping of prefixed tissues followed by staining are methods of choice for greater cellular resolution.

Receptor autoradiography has been used for both in vivo and in vitro studies. In the next sections, advantages and disadvantages of both techniques are summarized.

In vivo receptor autoradiography

Among the various receptor autoradiographic methods currently available, in vivo receptor autoradiography was the first to be used by many investigators (for reviews, see Kuhar & Yamamura, 1975; Kuhar, 1993). This technique involves the administration of a radioligand via a systemic or intracerebral route into living animals, and subsequent detection of radioactivity using autoradiography. Under strictly controlled conditions, the macroscopic (organs), cellular (cell types), or subcellular (organelles) distribution of the radiolabelled probe can be achieved. We recently used this technique to demonstrate the unique distribution of sigma receptor sites in the mouse brain. Using (+)-[3H]SKF 10 047 as radioligand, the highest levels of sigma labelling were found in various cranial nerve nuclei, whereas lower but still significant amounts of labelling were observed in the cortex, hippocampus, various hypothalamic nuclei, red nucleus, substantia nigra, central grey, and cerebellum (Bouchard et al., 1996). This in vivo distribution of sigma receptor sites corresponds rather well to that reported previously by others using a similar method (Compton et al., 1987; Weissman et al., 1990), or using membrane binding assays (Compton et al., 1987).

Theoretically, in vivo receptor labelling offers binding conditions similar to those prevailing in the physiological state, and it is compatible with perfusion fixation of target tissue, thus providing high morphological resolution. While in vivo autoradiography is certainly appealing for the anatomical or cellular localization of receptor sites, this technique has some limitations. This method requires radioligands that are metabolically stable and that fulfil the above mentioned criteria of irreversibility and that, in the case of CNS studies, these molecules should be able to cross the blood-brain barrier. Moreover, in order to overcome potential in vivo degradation, the injection of large quantities of radioligand is required. With this approach, it is also impossible to have direct control of the labelling conditions. Notwithstanding these issues, this method provides a basis for the development of non-invasive imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography scanning (SPECT). These latter methods use in vivo labelling to visualize receptor binding sites as well as to measure other parameters in the living human CNS (for example, see Kuhar et al., 1986; Frost and Wagner, 1990).