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Target site occupancy: Emerging generalizations from clinical and preclinical studies ☆

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ABSTRACT

What percentage of receptors, ion channels or transporters must be occupied by drugs to trigger therapeutic effects in patients, or by drugs and other ligands to induce physiological effects in humans or animals? Human studies utilizing Positron Emission Tomography and Single Photon Emission Computed Tomography, along with data from an array of preclinical methodologies, have begun to provide consistent answers to this question. The required target occupancy is dependent upon the molecular class of both target and ligand, and appears to be similar for both patient therapy and human or animal physiology. In the case of antagonists, approximately 60–90% target occupancy is required for G protein-coupled receptors, neurotransmitter transporters, and ligand-gated ion channels. Effective doses of agonists occupy a wider range of their target sites, dependent upon the intrinsic activity of the agonist, the receptor or ion channel reserve of the target site, and the response that is measured, with low efficacy agonists generally requiring high degrees of occupancy while high efficacy agonists generally require low degrees of occupancy. Target desensitization, competition by endogenous ligands, and regional target differences all influence target occupancy requirements. Measurements of target occupancy can help assure proper dosing and targeting of compounds in preclinical and clinical drug development as well as in basic research. Target occupancy generalizations can be especially important in establishing initial dosing recommendations for the many new drug targets provided by genomic and proteomic initiatives, where little data is available on their functional responses.

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1. Introduction

The ultimate targets of most drugs are specific binding sites on receptors, ion channels, transporters and enzymes. Recently, data have been accumulating on the drug occupancy levels required at these target sites in order to achieve therapeutic efficacy in humans, and on the drug or ligand occupancy levels required for the activation of biological responses in animal studies. The noninvasive methodologies of Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) tracer studies can be particularly informative as they have the sensitivity required to perform target occupancy studies in both humans and animals,

Abbreviations: BZD, benzodiazepine; CNS, central nervous system; DAT, dopamine transporter; EEDQ, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; EPS, extrapyramidal side effects; GPCR, G protein-coupled receptor; K_{ATP} channel, ATP-sensitive potassium channel; MAO-A, monoamine oxidase A; MAO-B, monoamine oxidase B; NAM, negative allosteric modulator; nAChR, nicotinic acetylcholine receptor; NET, norepinephrine transporter; NPA, N-propylnorapomorphine; PDE-4, cAMP-specific phosphodiesterase-4; PAM, positive allosteric modulator; PET, Positron Emission Tomography; SERT, serotonin transporter; SPECT, Single Photon Emission Computed Tomography.

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allowing for the quantification of a specifically bound compound, in both normal and diseased tissues (for reviews see: van Waarde, 2000; Klimas, 2002; Passchier et al., 2002; Talbot & Laruelle, 2002; Brooks, 2005; Heiss & Herholz, 2006; Lee & Farde, 2006). Clinical and preclinical assays of target site occupancy by drugs and new drug candidates can provide optimal dosing of the desired target site with minimal side effects, and promise to reorient drug discovery and development to the achievement of specified levels of target occupancy which can most effectively stimulate or inhibit specific receptors, ion channels, transporters and enzymes. Similarly, target occupancy measurements can improve animal research studies by optimizing efficacy at the desired target site while minimizing off-target effects.

This review discusses some emerging generalizations about the degree of in vivo occupancy needed for the effective activation or blockade of physiological and behavioral responses by drugs, ligands and endogenous agonists. Human target occupancy data (by PET and SPECT) are now available for many G protein-coupled receptors (GPCRs), for select neurotransmitter transporters, and for some enzymes and ion channels, providing valuable information on the target occupancy required for efficacy at precedented targets. These data can be extended to build a rationale for predicting clinical efficacy for novel chemical entities acting at both precedented and unprecedented targets. In addition, time-dependent animal and clinical target occupancy data can provide precise information about the onset, steady state and decline of ligand and drug effects that is more direct than determinations based upon plasma drug levels. For clinical disease states without well-validated preclinical disease models, and for receptor or ion channel targets with poorly understood or unknown physiological actions, emerging generalizations on target occupancy requirements can guide both preclinical pharmaceutical research and basic science research to achieve effective levels of target occupancy for activation or inhibition of any resulting physiological actions.

As discussed in this review, some answers are beginning to appear to key molecular target questions such as: What degree of target site occupancy is needed for biological responses? Do the requirements differ for ion channels, transporters and different classes of receptors? Are the requirements the same for agonists, partial agonists and antagonists? Do target site occupancies for animal behavioral and physiological responses correlate with human therapeutic actions of drugs? Do closely related molecular targets (e.g. antagonism of GPCRs) require similar degrees of target site occupancy for therapeutic action in different disease states? Such target occupancy information is important both in early drug discovery, where accurate knowledge of the degree of occupancy could help determine the suitability of a drug candidate for further development, and later in the drug development process, when molecular target site occupancy measurements can guide dosing selection and clinical trial design. For example, initial dose-ranging studies of early clinical trials often require large group sizes to obtain statistically significant results using symptom evaluations as the primary outcome. These early, dose-ranging clinical trial group sizes may be reduced, and development timelines shortened, if a change can be made from primary outcome measures to a surrogate marker defined by the target occupancy level needed to affect a preclinical disease model or to activate a preclinical biological response. In addition, many clinical trials have ended with no evidence of efficacy but also without knowing whether adequate levels of drug ever reached the intended target site. A target occupancy focus may provide evidence that the target site was occupied by the drug candidate to the intended extent, and thus that an adequate test of its therapeutic potential was performed. If the emerging generalizations on target occupancy requirements prove sufficiently useful, then newly-discovered targets from genomics and proteomics initiatives may be dosed according to their target-class occupancy requirements for initial evaluation in preclinical disease models, even without knowledge of their normal biological actions. Improved guidance of both discovery and development processes by target occupancy data could significantly shorten and improve new drug development. Molecular target occupancy data also provide important insights into physiological activation by endogenous agonists, and into quantitative requirements for ligand dosing in basic animal research. In vivo target occupancy measurements can also help answer critical questions about brain activation, such as the effects of receptor reserve (spare receptors) (Hoyer & Boddeke, 1993; Zhu, 1993; Pliska, 1999), about regional differences in target site responses (Cox & Waszczak, 1990; Claustre et al., 2003; Chou et al., 2006), about the effects of disease processes and disease progression on receptors (Hoffman & Donovan, 1995; Shinotoh et al., 2004; Brooks, 2005; Lee & Farde, 2006), about possible differences between clinical responders and non-responders in the degree of target occupancy (van Waarde, 2000) and about the degree of receptor occupancy by endogenous agonists in animals and humans (Laruelle, 2000; Frankle et al., 2004). This review will focus on these issues, primarily based upon human target occupancy studies for Central Nervous System (CNS) drugs, with additional primate and animal data added wherever the human data are sparse. Studies on membrane preparations and transfected cells will not be reviewed since membrane preparations can exhibit altered receptor properties compared to living cells (Emerit et al., 1991; Bylund & Toews, 1993; Kenakin, 1993) and transfected systems often over-express recombinant receptors at high densities that lead to a high receptor reserve and distortions in agonist efficacy (Adham et al., 1993; Hoyer & Boddeke, 1993; Whaley et al., 1994; Kenakin, 1997). Although this review is focused primarily on CNS therapeutics, the same molecular target classes (GPCRs, ion channels, transporters, enzymes) are often attractive to other therapeutic fields (e.g. cardiovascular, endocrine, urological etc.) and similar target occupancy approaches can be applied. Previous reviews of target site occupancy have been published, focused primarily on PET and SPECT techniques (van Waarde, 2000; Passchier et al., 2002; Jones, 2005; Wang & Maurer, 2005). For this reason, technical aspects of those methodologies will not be reviewed.

2. How much target occupancy is required?

2.1. G protein-coupled receptor antagonists

The largest published dataset providing a clear link between receptor occupancy measurements and therapeutic efficacy is that of the antipsychotics. A representative PET dataset, obtained using either [^{11}C]raclopride (Farde et al., 1992; Nyberg et al., 1995, 1996a, 1996b) or [^{123}I]epidepride (Bigliani et al., 1999) imaging, showing the occupancy of human striatal D2/D3 receptors by *typical* antipsychotics at therapeutic doses, is collated in Fig. 1A. All mean occupancies for typical antipsychotics were in the range of 61–82% D2/D3 occupancy with an overall mean occupancy of 74% (Fig. 1A). Fig. 1B shows a representative dataset for the occupancy of human striatal D2/D3 receptors by *atypical* antipsychotics at therapeutic doses. In addition to [^{11}C]raclopride data (Farde et al., 1992, 1994, 1995; Kapur et al., 1995; Nordstrom et al., 1995; Nyberg et al., 1996a; 1997; Nordstrom et al., 1998; Kapur et al., 1999; Nyberg et al., 1999, 2002; Talbot & Laruelle, 2002; Bressan et al., 2003b) and [^{123}I]epidepride data (Bigliani et al., 2000; Stephenson et al., 2000; Bressan et al., 2003a; Stone et al., 2005), [^{76}Br]Br lisuride (Martinot et al., 1996), [^{123}I]IBZM (Frankle et al., 2004) and [^{11}C]FLB457 (Nyberg et al., 2002) data are also included. Atypical antipsychotics exhibited an overall mean dopamine receptor occupancy of 64% (Fig. 1B). These datasets show a statistically significant difference in striatal dopamine receptor occupancy by typical vs. atypical antipsychotics ($p<0.002$, two tailed t-test), in agreement with the hypothesis that atypical antipsychotics require lower occupancy for therapeutic efficacy (Kapur, 1998; Kasper et al.,