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Neuropharmacology 49 (2005) 220–229
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

In vivo labelling of α5 subunit-containing $GABA_A$ receptors using the selective radioligand [^{3}H]L-655,708

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Received 7 October 2004; received in revised form 12 February 2005; accepted 7 March 2005

Abstract

L-655,708 is an imidazobenzodiazepine possessing 30–70-fold selectivity for the benzodiazepine binding site of $GABA_A$ receptors containing an α5 rather than α1, α2 or α3 subunit. In the present study, [^{3}H]L-655,708 was used to label mouse brain benzodiazepine binding sites in vivo. When compared to inhibition of in vivo binding of the non-selective ligand [^{3}H]Ro 15-1788, the pharmacology of mouse in vivo [^{3}H]L-655,708 binding was consistent with selective in vivo labelling of α5 subunit-containing $GABA_A$ receptors. Thus, diazepam was equipotent at inhibiting in vivo [^{3}H]L-655,708 and [^{3}H]Ro 15-1788 binding; zolpidem, which has very low affinity for α5-containing $GABA_A$ receptors, gave no inhibition of in vivo [^{3}H]L-655,708 binding despite inhibiting in vivo [^{3}H]Ro 15-1788 binding; and L-655,708 was more potent at inhibiting the in vivo binding of [^{3}H]L-655,708 compared to [^{3}H]Ro 15-1788. This pharmacological specificity of in vivo [^{3}H]L-655,708 binding was confirmed autoradiographically. Hence, the anatomical distribution of in vivo [^{3}H]L-655,708 binding was comparable to the distribution of α5-containing $GABA_A$ receptors identified in vitro. Moreover, this distribution was distinct from that identified using [^{3}H]Ro 15-1788. These data therefore suggest that [^{3}H]L-655,708 can be used to identify α5-containing $GABA_A$ receptors in vivo and that this ligand can be used to measure receptor occupancy of α5-selective ligands.

Keywords: $GABA_A$ receptors; α5 subunit; Benzodiazepine; In vivo binding; [^{3}H]L-655,708

1. Introduction

Mammalian $GABA_A$ receptors are generally accepted to be pentameric assemblies of subunits derived from the 19 members of the $GABA_A$ receptor subunit family (α1–6, β1–3, γ1–3, δ, ε, θ, π and ρ1–3) (Barnard et al., 1998; Bonnert et al., 1999) with the majority of native receptors containing α, β and γ subunits in a 2:2:1 stoichiometry (Sieghart and Sperk, 2002). In addition to playing a major role in mediating the effects of GABA, the main inhibitory neurotransmitter within the CNS, the $GABA_A$ receptor also contains a number of modulatory sites that allosterically modify receptor function and are thought to be responsible for the pharmacological and clinical effects of a diverse range of compounds including barbiturates, certain anaesthetics, neurosteroids and benzodiazepines (Sieghart, 1995; Korpi et al., 2002).

The pharmacology of $GABA_A$ receptors that possess a benzodiazepine binding site is dictated by the α and γ subunits contained within the receptor, since the binding site occurs at the interface of these two subunits

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0028-3908/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2005.03.004

(McKernan and Whiting, 1996). Furthermore, since the major γ subunit occurring in native receptors is γ2, heterogeneity in benzodiazepine pharmacology of $GABA_A$ receptors in the brain is primarily a function of the α subunit present (McKernan and Whiting, 1996). For example, non-selective benzodiazepines, such as diazepam, have equivalent affinity for the benzodiazepine binding site of $GABA_A$ receptors containing an α1, α2, α3 or α5 subunit yet have essentially no affinity for $GABA_A$ receptors containing an α4 or α6 subunit; a selectivity solely attributable to the presence of an arginine residue in the α4 and α6 subunit which replaces a histidine residue found in α1, α2, α3 and α5 (Wieland et al., 1992; Benson et al., 1998).

Non-selective benzodiazepines, such as diazepam, possess a number of behavioural properties (e.g. anxiolysis, sedation and anticonvulsant activity) and the heterogeneous distribution of mRNA and protein for the different α subunits within the brain suggests that there may be functional heterogeneity of benzodiazepine modulatory actions within the CNS (Wisden et al., 1992; Fritschy and Möhler, 1995; Pirker et al., 2000). In other words, particular behavioural properties of benzodiazepines may be associated with specific α subunit-containing subtypes of the $GABA_A$ receptor (Lüddens et al., 1995). Recently, transgenic mice in which particular α subunits have been rendered insensitive to diazepam by changing the histidine residue which is crucial for diazepam binding to an arginine (Benson et al., 1998) have been used to help delineate which $GABA_A$ receptor subtypes are associated with which of the pharmacological properties of diazepam (Rosahl, 2003; Rudolph and Möhler, 2004). For example, α1-containing $GABA_A$ receptors are involved in benzodiazepine-induced sedation (Rudolph et al., 1999; McKernan et al., 2000), a role confirmed pharmacologically by the fact that L-838417, a compound which does not modulate the function of α1-containing receptors, demonstrates reduced sedative liability (McKernan et al., 2000) and zolpidem, which interacts relatively selectively with α1 subunit-containing $GABA_A$ receptors is hypnotic (Rush, 1998).

L-655,708 is an imidazobenzodiazepine which possesses greater affinity for α5 versus α1, α2 or α3 subunit-containing $GABA_A$ receptors (Quirk et al., 1996). [^{3}H]L-655,708 has been used to show autoradiographically that α5 subunit-containing $GABA_A$ receptors have a high level of expression in the rat and human hippocampus (Sur et al., 1999; Howell et al., 2000; Li et al., 2001), in agreement with in situ hybridization and immunohistochemical studies (Wisden et al., 1992; Fritschy and Möhler, 1995; Pirker et al., 2000). The function of α5-containing $GABA_A$ receptors is not well understood, although their relatively high expression in the hippocampus implicates this particular subtype in hippocampal-dependent processes such as cognition, a role supported by recent evidence from α5 knock-out and diazepam-insensitive transgenic mice (Collinson et al., 2002; Crestani et al., 2002) as well as compounds with α5 selectivity (Chambers et al., 2002, 2003; Sternfeld et al., 2004). More recently, this $GABA_A$ receptor subtype has also been implicated in the mechanism of pre-pulse inhibition (Hauser et al., 2005) as well as tolerance to the sedative actions of diazepam (van Rijnsoever et al., 2004).

In addition to L-655,708 a number of other imidazobenzodiazepines with binding selectivity for α5- over α1-, α2- and α3-containing $GABA_A$ receptors have also been described. These include not only Ro 15-4513 (Hadingham et al., 1993) but also RY 80 (Liu et al., 1996; Skolnick et al., 1997; Opacka-Juffry et al., 1999; Li et al., 2001), RY 023 (Liu et al., 1996; June et al., 2001) and RY 024 (Vergnes et al., 2001; Bailey et al., 2002; McKay et al., 2004). Clearly, such compounds represent valuable tools for further elucidating the functions of the α5 subtype. However, in order to attribute specific behavioural effects of subtype selective compounds to particular $GABA_A$ receptor populations it is necessary to be able to discriminate between the levels of occupancy at different $GABA_A$ receptor populations in vivo. Thus, although the in vivo binding of radiolabelled Ro 15-1788 has been used to establish the relationship between receptor occupancy and intrinsic efficacy of non-selective benzodiazepines, not only in animals (Facklam et al., 1992) but also in man (Malizia and Richardson, 1995), the non-selective binding affinity of Ro 15-1788 means that it cannot be used as a radioligand to selectively determine the occupancy of a binding-selective compound at a particular $GABA_A$ subtype. Therefore, in the present study we evaluated whether [^{3}H]L-655,708 is suitable for specifically labelling α5 subunit-containing $GABA_A$ receptors in vivo in a manner analogous to that used to measure occupancy of the combined α1, α2, α3 and α5 subunit-containing $GABA_A$ receptor population using [^{3}H]Ro 15-1788 (Atack et al., 1999). Pharmacological and autoradiographic characterisation of the in vivo binding properties of [^{3}H]L-655,708 were used to establish that this ligand bound specifically to $GABA_A$ receptors containing an α5 subunit.

2. Materials and methods

2.1. Drugs

[^{3}H]L-655,708 was synthesised in-house as described elsewhere (Quirk et al., 1996). This compound is also commercially available from American Radiolabelled Chemicals, Inc. [^{3}H]Ro 15-1788 (70–87 Ci/mmol) was purchased from NEN (PerkinElmer Life Sciences, Boston, MA). Diazepam, flunitrazepam and zolpidem