

The proconvulsant effects of the GABA_A α 5 subtype-selective compound RY-080 may not be α 5-mediated

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Abstract

RY-080 (ethyl 8-ethynyl-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate) is an imidazobenzodiazepine with 40–50-fold higher affinity for the benzodiazepine binding site of α 5- rather than α 1-, α 2- or α 3-containing GABA_A receptors. Previous data describing RY-080 as being convulsant suggests that inverse agonists selective for the α 5 subtype may not be suitable for clinical development. In the present study, we show that RY-080 possesses inverse agonism for the α 1 and α 5 subtypes of human recombinant GABA_A receptors and whilst not convulsant it was proconvulsant. Hence, with pentylenetetrazole alone, the dose predicted to give tonic convulsions in 50% of the mice (ED₅₀) was 108 mg/kg whereas in the presence of 1 and 10 mg/kg RY-080, the ED₅₀s were 93 and 57 mg/kg, respectively. In vivo [³H]L-655,708 and [³H]Ro 15-1788 binding assays showed that the subtype selectivity of RY-080 in vivo was 7–10-fold for α 5-relative to α 1- and α 2/ α 3-containing receptors (respective ID₅₀ values of 0.93, 9.7 and 6.2 mg/kg) and is therefore much lower than seen in vitro. Consequently, it is not possible to define a dose of RY-080 which gives high occupancy of the α 5 subtype without binding to other subtypes and accordingly the proconvulsant effects of RY-080 cannot be attributed solely to the α 5 subtype.

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Keywords: GABA_A receptor; α 5 subunit; Benzodiazepine; RY-080; Proconvulsant; In vivo binding

1. Introduction

Mammalian GABA_A receptors are ligand-gated chloride ion channels comprising of pentameric assemblies of subunits from a 19-member gene family (α 1–6, β 1–3, γ 1–3, δ , ϵ , θ , π and ρ 1–3; Simon et al., 2004). The majority of GABA_A receptors contain α , β and γ 2 subunits arranged in an $\alpha\beta\alpha\beta\gamma$ sequence in a clockwise direction as viewed from the synapse (Minier and Sigel, 2004).

In addition to GABA binding sites, which occur at the interface of α and β subunits, most GABA_A receptors also contain a recognition site for prototypic benzodiazepines (exemplified by diazepam) which is found at the interface of the γ 2 and either an α 1, α 2, α 3 or α 5 (but not α 4 or α 6) subunit (Sieghart and Sperk, 2002). Of these four subtypes, the α 1 β γ 2 combination predominates in the brain, followed by α 2-then α 3-containing

receptors with the α 5 subtype being least abundant (McKernan and Whiting, 1996; Sieghart and Sperk, 2002). Despite its relatively low level expression in the whole brain, the α 5 subtype has a highly heterogeneous pattern of expression, most notably high levels of expression within the hippocampus (Wisden et al., 1992; Fritschy and Mohler, 1995; Pirker et al., 2000) where it constitutes around 25% of the total GABA_A/benzodiazepine receptor population (Sur et al., 1998, 1999). The highly localized expression of this subtype suggests that it is associated with specific hippocampal functions and indeed these receptors are involved in aspects of learning and memory (Collinson et al., 2002; Crestani et al., 2002). Consequently, this receptor population is an attractive target for potential cognition enhancers (Chambers et al., 2004). More specifically, since non-selective agonists impair and non-selective inverse agonist enhance cognition, it is proposed that an inverse agonist selective for the α 5 subtype should enhance cognition (Maubach, 2003) but be devoid of the anxiogenic and proconvulsant liabilities that limit the clinical utility of non-selective inverse agonists such as FG 7142

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(*N*-methyl- β -carboline-3-carboxamide; Horowski and Dorow, 2002).

A number of structurally-related imidazobenzodiazepines with 25–100-fold higher affinity for $\alpha 5$ compared to $\alpha 1$, $\alpha 2$ or $\alpha 3$ subunit-containing GABA_A receptors have been described, including L-655,708 (ethyl (*S*)-[11,12,13,13a-tetrahydro-7-methoxy-9-oxo]-[⁹H]-imidazo[1,5-*a*]pyrrolo[2,1-*c*][1,4]benzodiazepine-1-carboxylate; Quirk et al., 1996), RY-010, -023, -024 and -080 (the ethyl 8-ethyl-, *t*-butyl 8-ethynyl-, *t*-butyl 8[(trimethylsilyl)ethynyl]- and ethyl 8-ethynyl-analogues of 5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate: Liu et al., 1995, 1996; Skolnick et al., 1997). Where measured, these compounds have inverse agonist efficacy at the $\alpha 5$ subtype (Liu et al., 1995; Kelly et al., 2002) and would therefore appear to be ideal compounds for evaluating the hypothesis that an $\alpha 5$ -selective inverse agonist might enhance cognition but be devoid of the anxiogenic and convulsant and/or proconvulsant liabilities associated with non-selective inverse agonists (Maubach, 2003). However, RY-023, -024 and -080 have all been reported to be convulsant or proconvulsant in mice (Liu et al., 1996). Moreover, these effects were proportional to the extent of $\alpha 5$ efficacy in so far as RY-024 has full inverse agonist efficacy at this subtype (Liu et al., 1995) and produced convulsions in 80% of mice whereas RY-023, which has partial inverse agonism at the $\alpha 5$ subtype (Liu et al., 1995) was only convulsant in 22% of mice (Liu et al., 1996). In the absence of details of the efficacy of these compounds at the other subtypes as well as the extent of the in vivo receptor occupancy at the $\alpha 5$ subtype (i.e., do doses that produce convulsions selectively occupy only $\alpha 5$ -containing receptors?) these data are difficult to interpret but clearly have implications for any attempts to develop $\alpha 5$ subtype selective cognition enhancers. Hence, it is important to establish whether inverse agonism at the $\alpha 5$ subtype is capable of producing convulsant or proconvulsant activity.

The purpose of the present study, therefore, was to characterise the intrinsic efficacy and assess the proconvulsant effects of RY-080, a compound with higher affinity for the $\alpha 5$ compared to $\alpha 1$, $\alpha 2$ or $\alpha 3$ subtypes (respective K_i values=0.5, 28, 21 and 26 nM; Liu et al., 1995, 1996; Skolnick et al., 1997) and convulsant activity (Liu et al., 1996). The efficacy of this compound at the $\alpha 5$ subtype has not previously been reported but given its structural similarity to RY-023 and RY-024 is assumed to possess inverse agonist efficacy at this subtype. Finally, the in vivo effects of RY-080 were related to occupancy at $\alpha 5$ versus $\alpha 1$, $\alpha 2$ and $\alpha 3$ -containing GABA_A receptors using [³H]L-655708 and [³H]Ro 15–1788 ([³H] 8-fluoro 5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid ethyl ester) in vivo binding assays, respectively.

2. Methods

2.1. Drugs

[³H]L-655708 was synthesised in-house as described elsewhere (Quirk et al., 1996). This compound is also now commercially available from American Radiolabelled Chemicals, Inc. [³H]Ro 15–1788 (70–87Ci/mmol) was purchased

from PerkinElmer Life Sciences, Boston, MA). Diazepam, flunitrazepam and zolpidem were obtained from RBI (Sigma-Aldrich, Gillingham, UK) and bretazenil was a gift from Roche Labs. RY-080 was prepared in a similar manner to that described previously (Liu et al., 1996).

2.2. Intrinsic efficacy of RY-080

Ovary tissue was removed from female adult *Xenopus laevis* anaesthetized by immersion in 0.4% 3-aminobenzoic acid ethylester. Stage V and VI oocytes were isolated using fine forceps and treated with collagenase to remove follicle cells. cDNAs encoding different human GABA_A receptor subunit expressed in either a pCDM8 or pcDNA1/Amp expression vector were directly injected into the nuclei of individual oocytes in 10–20 nl of injection buffer (88 mM NaCl, 1 mM KCl, 15 mM HEPES buffer, pH 7.0) at a concentration of 20 ng/ μ l. After 24–72 h., oocytes were placed in a 50 μ l bath perfused with modified Barth's medium, impaled with two 1–3 M Ω electrodes containing 2 M KCl and voltage clamped at –30 to –80 mV. RY-080 was dissolved in dimethylsulphoxide (DMSO) and tested at a concentration of around 500-fold the K_i (i.e., 10 and 0.25 μ M for $\alpha 1$ - and $\alpha 5$ -containing oocytes, respectively), with a DMSO concentration in the perfusate of 0.1%. The drug was preapplied for 30 s prior to the addition of a concentration of GABA that elicited a current 20% of the maximal response (EC_{20}) produced at a GABA concentration of 3 mM. The modulation of the EC_{20} was calculated as:

$$\left(\frac{\text{Current}_{\text{GABA } EC_{20} + \text{RY-080}} - \text{Current}_{\text{GABA } EC_{20}}}{\text{Current}_{\text{GABA } EC_{20}}} \right) * 100$$

2.3. In vivo studies

Male Swiss–Webster mice (23–30 g; B&K International, Hull, UK) were used in the convulsant, proconvulsant and in vivo binding experiments. Dose volumes for i.p. and s.c. injections were 10 μ l/g and for i.v. injections were 5 μ l/g. All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986 and associated guidelines.

2.3.1. Convulsant and proconvulsant activity of RY-080

2.3.1.1. Convulsant activity. Mice ($n=10$ /group) received injections of either RY-080 (40 and 100 mg/kg i.p. in 0.5% methyl cellulose vehicle) or pentylenetetrazole (PTZ; 40 or 100 mg/kg s.c. in isotonic saline). Immediately afterwards, mice were placed in perspex observation boxes and observed for 30 min during which time the number of animals undergoing tonic convulsions was noted.

2.3.1.2. Proconvulsant activity. Mice (25–28 g) were pretreated for 15 min with vehicle (0.5% carboxy methylcellulose) or RY-080 (10, 20 and 40 mg/kg i.p.) following which a subthreshold dose of PTZ was injected (40 mg/kg. s.c.). Animals were then observed for 30 min as described above. In a

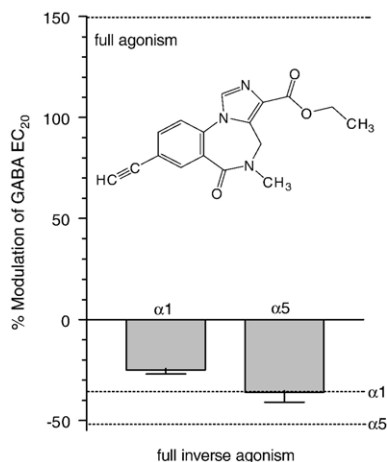


Fig. 1. Efficacy and structure of RY-080. Efficacy of RY-080 was measured against human recombinant GABA_A receptors transiently expressed in *Xenopus laevis* oocytes and containing $\beta 2$ plus $\gamma 2$ and either an $\alpha 1$ or $\alpha 5$ subunit. Positive values represent an increase and negative values a reduction of the current produced by the application of RY-080 (10 and 0.25 μ M at $\alpha 1$ and $\alpha 5$ -containing receptors, respectively) plus an EC₂₀-equivalent of GABA compared to a GABA EC₂₀ alone. Data shown are means \pm S.E.M. ($n=4-6$). Dashed lines indicate the extent of modulation at $\alpha 1$ - or $\alpha 5$ -containing receptors by the prototypic non-selective agonist DMCM (-36 and -52% , respectively; Sternfeld et al., 2004) and by non-selective agonists such as diazepam or chlordiazepoxide (c. $+150\%$).

separate experiment, mice received various doses of PTZ in the absence or presence of RY-080 (1 and 10 mg/kg i.p. in 0.5% carboxy methylcellulose or 10 mg/kg i.p. in 10% Emulphor:90% saline; Liu et al., 1996). From the resulting dose–response curves, estimates of the dose of PTZ required to produce convulsions in 50% of mice (ID₅₀) was interpolated.

2.3.2. In vivo binding of [³H]L-655,708 and [³H]Ro 15–1788

The inhibition of the in vivo binding of [³H]L-655,708 and [³H]Ro 15–1788 was used to measure the extent to which RY-080 occupied the benzodiazepine binding site of $\alpha 5$ - and $\alpha 1$ -, $\alpha 2$ - and $\alpha 3$ -containing GABA_A receptors, respectively, essentially as described elsewhere (Atack et al., 1999, 2005). In brief, mice received given i.p. injections of either vehicle (0.5% carboxy methylcellulose) or various doses of RY-080 followed by either [³H]L-655,708 or [³H]Ro 15–1788. One or 3 min later (for [³H]L-655,708 or [³H]Ro 15–1788, respectively) animals were killed by stunning and decapitation to give a total RY-080 pre-treatment time of 15 min. In the case of [³H]L-655,708 the brain stem and cerebellum were removed to leave the forebrain ($\alpha 5$ -containing GABA_A receptors) whereas for animals receiving [³H]Ro 15–1788, the brain and spinal column were removed and the cerebellum ($\alpha 1$ -containing GABA_A receptors) dissected from the brain and the spinal cord ($\alpha 2/\alpha 3$ -containing GABA_A receptors) was removed from the spinal column using compressed air. For each radioligand, a separate group of animals were dosed with bretazenil (5 mg/kg i.p. in polyethylene glycol vehicle) to occupy at benzodiazepine binding sites and therefore define the extent of the non-specific binding of either [³H]L-655,708 or [³H]Ro 15–1788.

Tissues (forebrain, cerebellum or spinal cord) were weighed, homogenised in 10 vol of ice-cold homogenisation buffer and

then aliquots of homogenate (300 μ l for the forebrain and cerebellum and 200 μ l for the spinal cord) were filtered and washed over Whatman GF/B filter paper circles. Washed filters were then placed in scintillation vials, scintillation fluid added and then radioactivity in vials was measured using a Beckman LS 6500 scintillation counter.

For each tissue, radioactivity in bretazenil-treated animals (i.e., non-specific binding) was subtracted from the vehicle- and RY-080-treated animals to give specific binding. The specific binding in RY-080-treated animals was then expressed as a percent of that in vehicle animals. The extent to which specific binding is reduced relative to vehicle-treated mice reflects the degree to which RY-080 occupies the benzodiazepine binding site of the particular GABA_A receptor population. For example, if the in vivo binding in RY-080-treated animals is 35% of vehicle, then this represents an occupancy of benzodiazepine binding sites by RY-080 of 65%.

3. Results

3.1. Intrinsic efficacy of RY-080

The intrinsic efficacy of RY-080 was measured based on its ability to modulate the current produced by a GABA concentration which produced a response 20% of the maximum (EC₂₀) at human recombinant GABA_A receptors transiently expressed in *Xenopus laevis* oocytes (Fig. 1). At $\alpha 5$ -containing receptors, RY-080 decreased the GABA-induced current (i.e., was an inverse agonist) to an extent (-36%) similar to that produced by the non-selective full inverse agonist DMCM (methyl-6,7-dimethoxy-4-ethyl- β -carboline-3-carboxylate: -52% ; Sternfeld et al., 2004). RY-080 also had appreciable inverse agonist efficacy at the $\alpha 1$ subtype (-25%).

3.2. RY-080 is not convulsant

Mice dosed with either 40 or 100 mg/kg RY-080 showed no signs of convulsions (Fig. 2). However, when administered along with a dose of pentylenetetrazole (40 mg/kg) which in its

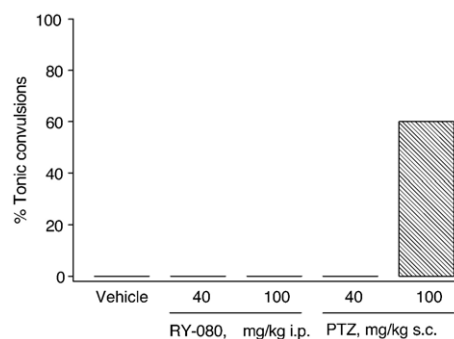


Fig. 2. RY-080 is not convulsant. Percentage of animals ($n=10$ /group) demonstrating tonic convulsions following treatment of RY-080 or PTZ. Neither 40 or 100 mg/kg RY-080 (i.p. in 0.5% carboxy methylcellulose) nor 40 mg/kg PTZ (s.c. in 0.9% saline) produced convulsions when administered alone. 100 mg/kg PTZ was included as a control and produced convulsions in 6 out of 10 mice (60%).

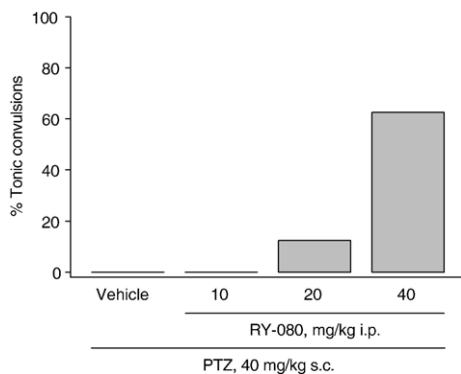


Fig. 3. Proconvulsant effects of RY-080. When administered in the presence of a dose of PTZ that does not produce convulsions (40 mg/kg), 40 mg/kg (but not 20 or 10 mg/kg) RY-080 produced convulsions ($n=8$ /group).

own right did not produce convulsions, RY-080 (40 mg/kg) did produce convulsions, indicating that a dose of 40 mg/kg is proconvulsant.

3.3. RY-080 is proconvulsant

Although neither 40 mg/kg PTZ nor 40 mg/kg RY-080 were convulsant in their own right (Fig. 2), when co-administered they produced convulsions in 5 out of 8 mice tested (Fig. 3). This proconvulsant effect of RY-080 was dose dependent since it was reduced or absent at doses of 20 and 10 mg/kg, respectively.

The proconvulsant effects of RY-080 were examined in more detail by examining the ability of RY-080 to shift the PTZ dose–effect curve (Fig. 4). This clearly shows that the proconvulsant effects of RY-080 (Fig. 3) are dependent upon the dose of RY-080. Hence, in the absence of RY-080, the ED_{50} for PTZ was 108 mg/kg but in the presence of 1 and 10 mg/kg RY-080 there was a leftward shift to give respective ED_{50} values of 93 and 57 mg/kg. When dosed in a 10% Emulphor vehicle (Liu et al., 1996), the potency of 10 mg/kg RY-080 ($ED_{50}=57$ mg/kg) was the same as when dosed in a carboxy methylcellulose vehicle.

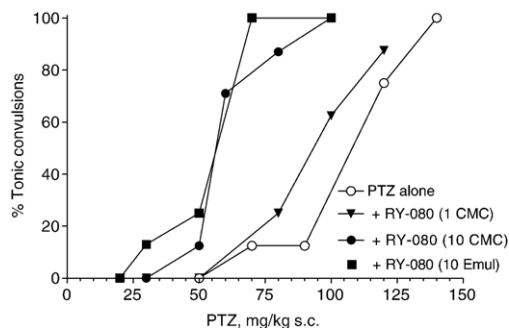


Fig. 4. Percentage of animals demonstrating tonic convulsions following s.c. administration of increasing doses (s.c. in 0.9% saline vehicle) of PTZ in the absence or presence of 1 or 10 mg/kg RY-080. Two different vehicle were used for the 10 mg/kg dose; 0.5% carboxy methylcellulose (CMC) and 10% Emulphor:90% saline (Emul.; Liu et al., 1996). There was a clear leftward shift of the dose–response curve in the presence of RY-080 consistent with this compound being proconvulsant. Values shown are the effect observed in groups of 8 mice.

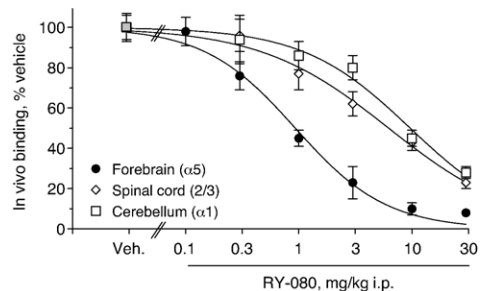


Fig. 5. Inhibition of the in vivo binding of [3 H]L-655708 to $\alpha5$ -containing $GABA_A$ receptors and [3 H]Ro 15–1788 to cerebellum ($\alpha1$) and spinal cord ($\alpha2/\alpha3$) subtypes by RY-080. Pre-treatment time=15 min and vehicle (veh.) was 0.5% carboxy methyl cellulose. Values shown represent mean \pm S.E.M. ($n=5-6$ /group).

3.4. Occupancy of $\alpha5$ - versus $\alpha1$ -, $\alpha2$ - and $\alpha3$ -containing $GABA_A$ receptors

In the in vivo binding assays, RY-080 gave dose-dependent inhibition of the binding of [3 H]L-655708 to $\alpha5$ -containing receptors in the forebrain and [3 H]Ro 15–1788 to $\alpha1$ - and $\alpha2/\alpha3$ -containing receptors in the cerebellum and spinal cord, respectively (Fig. 5). The doses giving 50% inhibition of in vivo binding to the $\alpha1$, $\alpha2/\alpha3$ and $\alpha5$ receptor populations were 9.7, 6.2 and 0.93 mg/kg, respectively whereas at a dose of 30 mg/kg high levels of occupancy at the $\alpha5$ subtype (92%), were accompanied by high levels of occupancy at the $\alpha1$ (72%) and $\alpha2/\alpha3$ (77%) subtypes. On the other hand, at a dose of 1 mg/kg, $\alpha5$ occupancy was 55% whereas corresponding values at the $\alpha1$ and $\alpha2/\alpha3$ subtypes were 14 and 23%.

4. Discussion

Given its structural similarity to other imidazobenzodiazepines with inverse agonist efficacy at the $\alpha5$ subtype, such as L-655708, RY-023 and RY-024 (Liu et al., 1995; Kelly et al., 2002), it is not surprising to find that RY-080 also has inverse agonist efficacy at this subtype. Moreover, RY-080 has full inverse agonist efficacy at the $\alpha5$ subtype and in that regard is more like RY-04 than RY-023 and L-655,708, the latter two of which have partial inverse agonism at the $\alpha5$ subtype (Liu et al., 1995; Kelly et al., 2002). In addition, the efficacy of RY-080 was also measured at the $\alpha1$ subtype, since this is the most abundant $GABA_A$ receptor population in the brain (McKernan and Whiting, 1996). At this subtype, RY-080 has appreciable inverse agonist efficacy and thus it is important to differentiate between the occupancy of RY-080 at $\alpha5$ and $\alpha1$ (as well as $\alpha2$ and $\alpha3$) receptor populations when interpreting the behavioural effects of this compound.

In the present study, RY-080 did not produce convulsions, even at a dose, 100 mg/kg, which exceed the dose calculated to produce convulsions in 50% of mice tested (CD_{50} ; 24 mg/kg; Liu et al., 1996). Although different vehicle were used in the present study (0.5% carboxy methyl cellulose) and that of Liu and colleagues (10% Emulphor:90% saline), this is unlikely to account for the discrepancy in convulsant activity since a side-by-side comparison of the proconvulsant activity of RY-080 in

the two different vehicles, showed that they produced similar results (Fig. 4).

Nevertheless, although not convulsant, RY-080 was proconvulsant in a dose-dependent manner (Figs. 3 and 4). Given that RY-080 is an $\alpha 5$ -selective compound, it would be tempting to ascribe this proconvulsant effect to this particular receptor population, with the implication that inverse agonists that selectively target these receptors would not be suitable for use in the clinic. However, when considering the receptor occupancy of RY-080 it is not possible to draw such a conclusion. Hence, the approximately 50-fold higher affinity of RY-080 for $\alpha 5$ - versus $\alpha 1$ -, $\alpha 2$ - or $\alpha 3$ -containing GABA_A receptors (K_i values of 0.5 and 21–28 nM) seen in vitro is not reflected in vivo, where there is a much reduced (7–10-fold) separation between the ID₅₀ values for the occupancy of $\alpha 5$ -compared to $\alpha 1$ - or $\alpha 2/\alpha 3$ -containing receptors (0.93 versus 6.2 and 9.7 mg/kg, respectively). A similar reduction in the in vivo versus in vitro $\alpha 5$ selectivity was also noted for L-655,708 (Atack et al., 2005) and while it is not clear why this is, it also occurs with the $\alpha 1$ -selective compounds zolpidem and CL 218872 (de la Sayette et al., 1991; Atack et al., 1999).

The consequence of this reduced in vivo $\alpha 5$ selectivity is that even at a low, proconvulsant dose of RY-080, there is appreciable occupancy of $\alpha 1$ -, $\alpha 2$ - and $\alpha 3$ -containing receptors. For example, whilst a dose of 1 mg/kg RY-080 gives $\alpha 5$ occupancy of 55%, there is still appreciable occupancy (14 and 23%) at the $\alpha 1$ and $\alpha 2/\alpha 3$ subtypes. Since these latter subtypes are much more abundant than the $\alpha 5$ subtype (Whiting and McKernan, 1996) it is possible that the proconvulsant liability is a consequence of this effects at the $\alpha 1$, $\alpha 2$ and/or $\alpha 3$ rather than the $\alpha 5$ subtype. Indeed, the much greater abundance of the $\alpha 1$ compared to $\alpha 5$ subtype (McKernan and Whiting, 1996) along with the appreciable inverse agonist efficacy of RY-080 at this subtype (Fig. 1) makes it a possible subtype for mediating the proconvulsant effects of this compound.

In summary, the present study shows that although the GABA_A $\alpha 5$ -selective compound RY-080 is not convulsant, it is, nevertheless, proconvulsant. The use of in vivo [³H]L-655,708 and [³H]Ro 15–1788 binding assays to measure occupancy at $\alpha 5$ or $\alpha 1$ and $\alpha 2/\alpha 3$ receptor populations suggest that the in vivo selectivity of RY-080 is less than that predicted by the in vitro binding affinities. Accordingly, at no dose of RY-080 was there appreciable occupancy at the $\alpha 5$ subtype in the absence $\alpha 1$, $\alpha 2$ or $\alpha 3$ subtype occupancy. Consequently, it is not possible to conclude that the proconvulsant effects of RY-080 are due to the $\alpha 5$ subtype; indeed, they may be due to the more abundant $\alpha 1$ subtype at which RY-080 also has inverse agonist efficacy. More generally, the studies described above highlight the need to take into account the extent to which $\alpha 5$ (or other subtype) binding selective compounds occupy different receptor populations otherwise the interpretation of in vivo data in terms of molecular mechanisms becomes difficult (e.g., Bailey et al., 2002).

Additional support for the possibility that inverse agonism at the $\alpha 5$ subtype does not cause convulsant or proconvulsant activity comes from studies using a compound, $\alpha 5$ IA (3-(5-Methylisoxazol-3-yl)-6-[(1-methyl-1,2,3-triazol-4-yl)methoxy]-1,2,4-triazolo[3,4-a]phthalazine), which has efficacy

rather than binding selectivity for the $\alpha 5$ subtype (i.e., it binds to $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ subtypes but only has significant efficacy at the $\alpha 5$ receptor; it is essentially an antagonist at the other subtypes). This compound was not proconvulsant nor did chronic dosing of the compound cause kindling (Dawson et al., in press). Taken together with the present study, these data suggest that compounds which have inverse agonism solely at the $\alpha 5$ subtype may indeed represent a novel therapeutic target that is devoid of the convulsant or proconvulsant liabilities associated with non-selective inverse agonists.

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