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L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for α5-containing $GABA_A$ receptors

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Abstract

The in vitro and in vivo properties of L-655,708, a compound with higher affinity for $GABA_A$ receptors containing an α5 compared to an α1, α2 or α3 subunit have been examined further. This compound has weak partial inverse agonist efficacy at each of the four subtypes but, and consistent with the binding data, has higher functional affinity for the α5 subtype. In a mouse hippocampal slice model, L-655,708 was able to enhance the long-term potentiation produced by a theta burst stimulation, consistent with a potential role for the α5 subtype in processes involving synaptic plasticity, such as learning and memory. When administered in a formulation specifically designed to achieve relatively constant plasma drug concentrations, and therefore maintain selective occupancy of α5- compared to α1-, α2- and α3-containing receptors (75 ± 4% versus 22 ± 10%, respectively), L-655,708 did not alter the dose of pentylenetetrazole required to induce seizures, indicating that the inverse agonist effects of L-655,708 at the α5 subtype are not associated with a proconvulsant liability. In the Morris water maze, L-655,708 enhanced performance not only during acquisition but also in a probe trial, demonstrating that this compound has cognition enhancing effects. These data further support the potential of α5-containing $GABA_A$ receptors as a target for novel cognition enhancing drugs.

1. Introduction

The GABA-gated $GABA_A$ receptor chloride channel is a pentameric arrangement of subunits derived from the $GABA_A$ receptor family (α1–6, β1–3, γ1–3, δ, θ, ϵ, π; Sieghart and Sperk, 2002; Simon et al., 2004) and is the target for a number of pharmacologically and clinically relevant drugs including barbiturates, neurosteroids, anaesthetics and benzodiazepines (Sieghart, 1995). The majority of $GABA_A$ receptors in the brain contain a benzodiazepine binding site (McKernan and Whiting, 1996) and such receptors contain β, γ2 and either an α1, α2, α3 or α5 subunit in a 2:2:1 stoichiometry (Sieghart and Sperk, 2002) with the benzodiazepine binding site occurring at the interface of the α and γ2 subunits (Sieghart, 1995). Classical benzodiazepines, such as diazepam, possess a variety of pharmacological actions including anxiolytic, sedating, myorelaxant, anticonvulsant and cognition-impairing activities and interact with α1-, α2-, α3- and α5-containing $GABA_A$ receptors with equivalent affinity and efficacy. Agonists and inverse agonists at the benzodiazepine binding site have opposite effects at the molecular level in that they increase and decrease, respectively, the GABA-stimulated flux of chloride through the GA BA_A receptor ion channel. These opposing effects of agonists and inverse agonists are manifested at the behavioural level by the fact that non-selective benzodiazepine agonists (i.e., those that enhance the functions of GABA at α1-, α2-, α3- and α5-containing $GABA_A$ receptors), such as flunitrazepam, diazepam and chlordiazepoxide, are anticonvulsant, anxiolytic and impair cognition (Curran, 1986, 1991) whereas non-selective inverse agonists, for example DMCM and FG 7142, increase vigilance and are proconvulsant and anxiogenic (Duka and Dorow, 1995;

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Rodgers et al., 1995; Sarter et al., 2001). These latter features mean that whilst non-selective inverse agonists might enhance cognition in animal models, they are unsuitable for clinical use (Dorow et al., 1983; Horowski and Dorow, 2002).

The distinct anatomical distributions of the different α subunits within the brain (Wisden et al., 1992; Pirker et al., 2000), suggest that there may be functional heterogeneity of benzodiazepine modulatory actions within the CNS, with particular pharmacological actions of, for example, diazepam, being associated with specific receptor subtypes (Rudolph and Möhler, 2004). With regard to $GABA_A$ receptors containing an α5 subunit, their preferential localization in the hippocampus (Wisden et al., 1992; Pirker et al., 2000), suggests that they play a role in hippocampally-mediated functions such as learning and memory (Maubach, 2003). More specifically, it has been hypothesised that a compound which is an inverse agonist selective for the α5 subtype may enhance hippocampally-based cognitive functions (Maubach, 2003). With respect to α5 selectivity, a number of compounds have been described which have 10–100-fold higher affinity for the α5 compared to α1, α2 or α3 subtypes, including Ro 15-4513, L-655,708, RY-010, RY-023, RY-024 and RY-080 (Liu et al., 1995, 1996; Quirk et al., 1996; Skolnick et al., 1997). For example, L-655,708 (FG 8094) is an imidazobenzodiazepine which possesses 50–100-fold selectivity for α5 versus α1, α2 or α3 subunit-containing $GABA_A$ receptors (Quirk et al., 1996). Moreover, where reported, these compounds have inverse agonist efficacy at the α5 subtype (Liu et al., 1995; Kelly et al., 2002) and in vivo RY-023, RY-024 and RY-080 have all been reported to be either convulsant or proconvulsant (Liu et al., 1996), indicating that it might not be possible to separate the cognition enhancing and proconvulsant effects since they could both be associated with the α5 subtype. These data contrast with that for the α5 efficacy-selective compound α5IA which was able to enhance cognition without any proconvulsant effects (Dawson et al., 2006). The reason for this discrepancy between binding- and efficacy-selective compounds is unclear. However, it is possible that RY-023, RY-024 and RY-080 may have inverse agonist efficacy at the α1, α2 and/or α3 as well as the α5 subtype and thus it is difficult to attribute the proconvulsant effects of these compounds solely to α5-containing receptors (Bailey et al., 2002).

In the present study we have further characterised the intrinsic efficacy of L-655,708 and shown it to enhance LTP in an in vitro mouse hippocampal slice model. Moreover, we used a slow release formulation (Atack et al., 2006b) to demonstrate that at plasma concentrations that produce relatively α5-selective occupancy (i.e., inverse agonist effects mediated via the α1, α2 and α3 subtypes are minimised) cognition enhancing effects can be achieved in the absence of a proconvulsant liability.

2. Materials and methods

2.1. Whole cell patch-clamp of L(tk⁻) cells

The intrinsic efficacy of L-655,708 was measured at human recombinant $GABA_A$ receptors containing β3 and γ2 subunits and either an α1, α2, α3 or α5 subunit expressed in mouse fibroblast L(tk⁻) cells (Hadingham et al., 1993). The cells were grown, and patch-clamping performed, as described previously (Dawson et al., 2006). The extent by which the current amplitude produced by an EC_{20}-equivalent concentration of GABA could be modulated by a 30 s pre-application of L-655,708 was measured and the modulation was calculated as follows:

$$\left(\frac{\text{Current}_{\text{GABA EC}_{20}+\text{L-655,708}} - \text{Current}_{\text{GABA EC}_{20}}}{\text{Current}_{\text{GABA EC}_{20}}}\right) \times 100$$

with negative values indicating that L-655,708 reduces the GABA EC_{20}-induced current (i.e., L-655,708 is an inverse agonist).

2.2. Brain slice electrophysiology

Long-term potentiation was evaluated in 350 μm thick parasagittal hippocampal slices prepared from C57 mice (6–9 months old, B&K Universal, Hull, UK) as described elsewhere (Dawson et al., 2006). In brief, slices ($n = 12$/group) were submerged in artificial CSF and the field excitatory post-synaptic potentials (EPSPs) were recorded in the stratum radiatum of the CA1 region following stimulation of the fibres in the Schaffer collateral-commissural path. Ten minutes after a stable baseline was established (characterised as a response that did not vary by more than 5% over a 30 min period), vehicle (0.1% DMSO) or L-655,708 (10 nM) was applied to the slices and 10 min later long-term potentiation was induced by a brief tetanus (10 stimuli at 100 Hz) followed 30 min later by a theta burst (four pulses at 100 Hz repeated 10 times with an interval of 200 ms; Seabrook et al., 1997). Data were analysed using regression analysis plus two-way analysis of variance with repeated measures (treatment and time).

2.3. Effects of L-655,708 in pentylenetetrazole proconvulsant assay

Male PVG rats (230–290 g; B&K Universal) received a subcutaneous implant of 60 mg pellets containing either vehicle (hydroxypropyl methylcellulose) or 1.5 mg L-655,708 ($n = 7$–8/group; Atack et al., 2006b) and 2.5 h later (i.e., after full recovery from the implantation procedure) pentylenetetrazole (PTZ; 40 mg/ml) was infused at a rate of 1 ml/min and the time of onset of either clonic or tonic convulsions noted. As a positive control, additional rats ($n = 8$) were given CGS 8216 (10 mg/kg i.p.) and 30 min later received infusions of PTZ as above. From the time taken to seizure and the infusion rate, the total dose of PTZ administered could be calculated: infusion rate (ml/s) × PTZ concentration (mg/ml) × (1000/weight g) × latency to seizure (s). For example, a latency to clonic seizure of 20 s in a 300 g rat corresponds to a PTZ dose of 44.4 mg.

A separate cohort of rats from the same batch as used for the PTZ assays were implanted with pellets of vehicle or L-655,708 ($n = 8$/group) as described above but after 2.5 h rather than administering PTZ they were given i.v. injections of either [^{3}H]L-655,708 or [^{3}H]Ro 15-1788 to measure the occupancy of α5- or α1-, α2- plus α3-containing $GABA_A$ receptors, respectively (Atack et al., 2006b). For each radioligand, an additional group of animals received bretazenil (5 mg/kg i.p. in polyethylene glycol 300) in order to define the extent of non-specific binding. One or three minutes after [^{3}H]L-655,708 or [^{3}H]Ro 15-1788 administration respectively, rats were killed by stunning and decapitation, brains were removed, the cortex dissected and then homogenised and processed as described previously (Atack et al., 2006b). The occupancy at the α5 and the combined α1, α2 plus α3 subtype populations was defined as the extent by which the specific binding of [^{3}H]L-655,708 and [^{3}H]Ro 15-1788 was reduced in L-655,708-implanted animals relative to vehicle animals. Trunk blood was collected following decapitation and the plasma L-655,708 concentrations were measured by HPLC with UV detection as described previously (Atack et al., 2006b).

2.4. Morris water maze

Male PVG rats (~300 g; B&K Universal) received subcutaneous pellets of either vehicle (60 mg high viscosity hydroxypropyl methylcellulose) or 1.5 mg

L-655,708 in the same vehicle as above and as described in more detail elsewhere (Atack et al., 2006b). Previous studies have shown that L-655,708 formulated in this manner maintains relatively constant plasma concentrations of around 100 ng/ml and that this corresponds to selective occupancy of α5- relative to α1-, α2- and α3-containing $GABA_A$ receptors (Atack et al., 2006b).

Two hours after pellet implantation, rats ($n = 8$/group) were placed in the Morris water maze which is a 2-m diameter tank containing opaque water and in which there is a 10 cm diameter platform, submerged 2 cm below the surface. The pool was surrounded by different shapes that served as spatial cues to aid locating the hidden platform and the movement of the rat within the pool was tracked using a video camera, the digitised image of which was analysed using HVS Water 2020 software (HVS Image, UK).

During the acquisition phase, each rat was given 9 blocks of 4 trials, with each block of trials separated by 0.5 h (Fig. 1). The block of 4 trials were performed within a 15 min period with each trial lasting a maximum time of 60 s. Once the platform had been located, the rat was allowed to remain on the platform for 30 s, after which time it was removed. Following an inter-trial interval of 30 s, the rat was placed back in the pool, in a different location relative to the platform. Fifteen minutes after completion of the acquisition phase of the experiment (i.e., the end of trial block 9), during which time the rat had presumably "learned" the position of the platform, a probe trial was carried out. During this part of the experiment, the hidden platform was removed and the time spent in the quadrant where the platform had previously been located (the target quadrant) was measured. The rationale for the probe trial was that if the rat had "remembered" the position of the platform, then it should spend more time in the target quadrant relative to the other quadrants. Alternatively, if the rat had no memory for the location of the platform, then it should, purely by chance, spend on average 25% of its time in the target quadrant.

3. Results

3.1. Intrinsic efficacy of L-655,708

Fig. 2 shows the effect of increasing concentrations of L-655,708 on the current produced by an EC_{20}-equivalent of GABA at different subtypes of the human $GABA_A$ receptor. The downward deflection of the concentration–effect curves show that at each subtype L-655,708 attenuated the GABA EC_{20}-evoked current. The maximal extent of this attenuation, i.e., inverse agonism (−11 to −23%; Table 1) was less than that seen with either the non-selective partial inverse agonist FG 7142 (−35 to −47%) or the non-selective full inverse agonist DMCM (−53 to −71%; Dawson et al., 2006). Thus, L-655,708 could be classified as a weak partial inverse agonist. However, L-655,708 clearly has selectivity for the α5 subtype in that the EC_{50} at this subtype (1.1 nM; Table 1) is considerably less than the functional affinity at the α1, α2 and α3 subtypes (320, 135 and 960 nM, respectively), although the relatively weak inverse agonism at these latter subtypes makes accurate determination of the EC_{50} difficult.

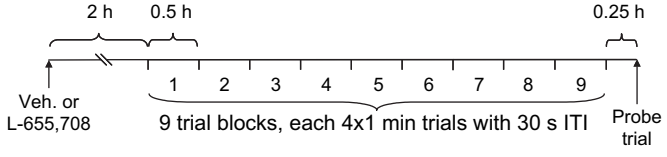

Fig. 1. Schematic representation of the Morris water maze experimental design. Two hours after receiving s.c. implantation of pellets of either L-655,708 or vehicle, rats were given 9 blocks of trials, each of which consisted of 4 trials, with a maximum duration of 1 min following which rats were allowed 30 s on the platform and were then removed before performing the next trial after an inter-trial interval (ITI) of 30 s. Each block of trials was started 0.5 h apart. Fifteen minutes after completion of the last trial, a 1 min probe trial was performed, during which the hidden platform was removed.

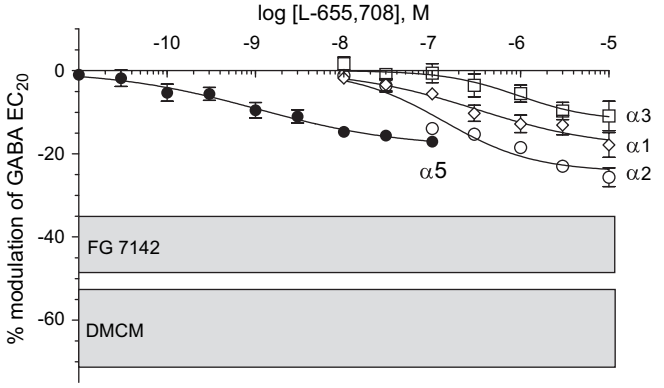

Fig. 2. L-655,708 is a weak partial inverse agonist with higher functional affinity for the α5 versus α1, α2 or α3 subtypes. The intrinsic efficacy of L-655,708 was measured at recombinant human $GABA_A$ receptors containing β3 and γ2 subunits and either an α1, α2, α3 or α5 subunit stably expressed in mouse fibroblast L(tk^-) cells. For comparison, shaded boxes illustrate the extent of inverse agonism seen with the non-selective partial inverse agonist FG 7142 (−35 to −47%) or the non-selective full inverse agonist DMCM (−53 to −71%; Dawson et al., 2006). Values shown are mean ± SEM ($n = 5$–7/data point).

3.2. L-655,708 enhances long-term potentiation in mouse hippocampal slices

The induction of long-term potentiation in hippocampal slices under control conditions (0.1% DMSO) as well as following application of L-655,708 (10 nM in 0.1% DMSO) is shown in Fig. 3. Under control conditions, the threshold stimulus (10 at 100 Hz) increased the EPSP rise times to levels 238 ± 22% of baseline (measured over the 5 min period following stimulation) and this remained significantly elevated even 30 min post-stimulation (202 ± 23%). The theta stimulation produced an even greater degree of increase with the EPSP rise times being 291 ± 28% of baseline 30 min post-stimulation.

At a concentration (10 nM) that should preferentially bind to α5-containing $GABA_A$ receptors, L-655,708 had no effect on baseline EPSPs and whilst there was an increase following threshold stimulation, the extent of this increase (to levels 30 min after stimulation that were 209 ± 20% of baseline)

Table 1
Binding and functional affinity and intrinsic efficacy of L-655,708 at human recombinant $GABA_A$ receptors

	Human $GABA_A$ receptors containing β3, γ2 plus			
	α1	α2	α3	α5
K_i, nM[a]	70 ± 9	48 ± 5	31 ± 5	1.0 ± 0.2
Modulation of GABA EC_{20}[b]	−18 ± 3% (7)	−23 ± 3% (7)	−11 ± 4% (6)	−17 ± 1% (5)
EC_{50}, nM[c]	320	135	960	1.1

[a] Data from Atack et al. (2005).
[b] Mean ± SEM of modulation observed in each individual cell (figures in parentheses = n).
[c] Calculated from the curve fitted through the mean data.

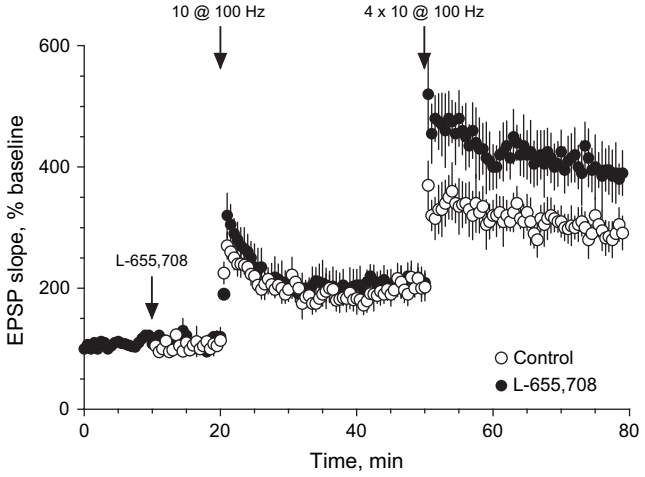

Fig. 3. L-655,708 enhances LTP following theta (4 × 10 events at 100 Hz) stimulation but not following threshold (10 events at 100 Hz) stimulation. Extracellular postsynaptic potentials (EPSPs) were recorded from the CA1 region of mouse hippocampal slices following stimulation of the Schaffer collateral pathway. L-655,708 (10 nM) was applied to slices 10 min after stable baseline conditions had been established. Values shown are mean ± SEM ($n = 12$/group).

did not differ from control (202 ± 23%). However, after theta stimulation, the population of EPSP rise times was significantly greater than under control conditions (395 ± 48% versus 291 ± 28%).

3.3. L-655,708 is not proconvulsant at an α5-selective dose

There was a significant effect of treatment on the threshold of PTZ required to produce clonic [$F(2,20) = 5.86$, $p < 0.01$] and tonic seizures [$F(2,20) = 6.77$, $p < 0.01$]. More specifically, when PTZ was infused 2.5 h after implantation of subcutaneous pellets of L-655,708, there was no significant effect on the dose of PTZ required to produce either clonic or tonic convulsions (Fig. 4). In contrast, prior treatment with CGS (10 mg/kg i.p.) significantly reduced ($p < 0.05$, Dunnett's t-test) the dose of PTZ required to produce both clonic and tonic seizures, consistent with the previously reported proconvulsant effects of this compound (Bennett, 1987). In order to confirm that sufficient compound had penetrated into the brain, L-655,708 occupancy was measured in a separate cohort of animals that were implanted at the same time as those used for the PTZ study (Fig. 5). In these animals, L-655,708 was found to give higher occupancy at the α5 subtype relative to the combined population of α1, α2 plus α3 subtypes (75 ± 4% versus 22 ± 10%), with plasma drug concentrations, 112 ± 13 ng/ml, being consistent with previous values (Atack et al., 2006b). Hence, the lack of a proconvulsant effect of L-655,708 was not due to insufficient occupancy of the α5 subtype.

3.4. L-655,708 improves learning and memory in the Morris water maze

Fig. 6A shows the escape latencies recorded during the acquisition phase of the study. An analysis of variance showed that there was an effect of treatment [$F(1,16) = 5.81$, $p < 0.03$] and an effect of trial [$F(8,128) = 14.34$, $p < 0.001$]. A comparison of the escape latencies of each 4-trial block showed that L-655,708-treated rats found the platform significantly quicker than vehicle-treated rats during trial blocks 6, 8 and 9 ($p < 0.05$). For example, although both vehicle- and L-655,708-treated animals had a similar latency to find the platform during trial block 1 (respective latencies = 50 ± 4 and 48 ± 4 s) by the end of trial block 9, L-655,708-treated animals found the platform much quicker (latency = 19 ± 4 s) than their vehicle-treated counterparts (latency = 35 ± 5 s).

With respect to the probe trial (Fig. 6B), an analysis of variance showed that there was a significant effect of treatment ($F[1,16] = 4.88$, $p < 0.05$). Post-hoc analyses showed that the time that vehicle-treated animals spent in the quadrant, 31 ± 2%, was significantly ($p < 0.05$) greater than chance alone (25%), indicating that they had remembered the position of the platform to some extent. However, this "memory" of the platform position was significantly greater in L-655,708-treated rats, which spent 39 ± 3% of their time in the quadrant.

4. Discussion

The fact that L-655,708 has inverse agonist efficacy at all four $GABA_A$ receptor subtypes with a BZ binding site is an extension of previous observations that it has inverse agonism of −16% against human recombinant α5-containing $GABA_A$ receptors transiently expressed in *Xenopus laevis* oocytes

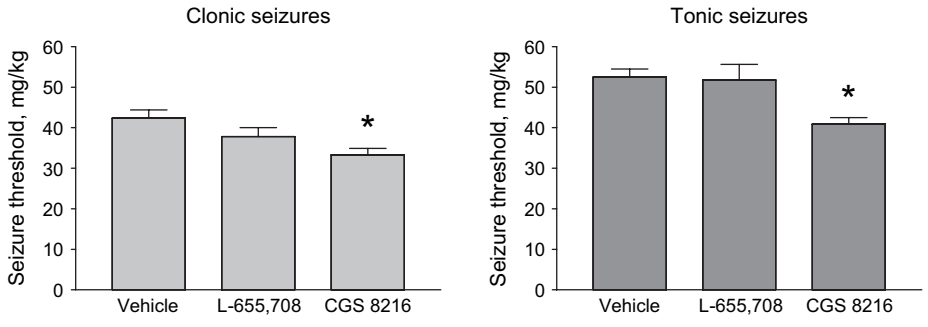

Fig. 4. L-655,708 does not alter the threshold to produce pentylenetetrazole-induced seizures in rat. The partial agonist CGS 8216 lowers the dose of PTZ required to produce both clonic and tonic seizures. Values shown are mean ± SEM ($n = 7$–8/group). *$p < 0.05$ Dunnett's t-test.

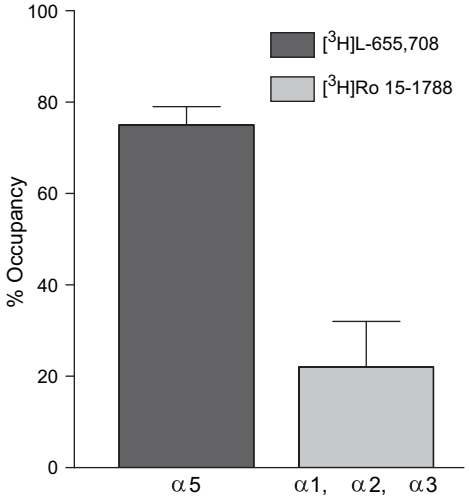

Fig. 5. Occupancy of the α5 subtype compared to the combined α1, α2 and α3 subtype population following subcutaneous implantation of a 60 mg pellet containing 1.5 mg of L-655,708 as measured using in vivo binding of [^{3}H]L-655,708 and [^{3}H]Ro 15-1788, respectively. The higher affinity for α5 containing receptors seen in vitro is reflected by higher occupancy at the α5 subtype (75 ± 4%) relative to the α1 plus α2 plus α3 subtypes (22 ± 10%). Values shown are mean ± SEM (n = 4/group).

(Kelly et al., 2002). The extent of the α5 inverse agonism previously reported in transiently expressed receptors (−16%; Kelly et al., 2002) is very similar to the degree of inverse agonism observed in the present study against stably expressed receptors (−17%; Table 1) and further emphasizes the similarity of efficacy measurements made using these two expression systems (Dawson et al., 2006). Given the structural similarities between L-655,708 and RY-010, RY-023, RY-024 and RY-080 and the fact that all these compounds have inverse agonist efficacy at the α5 subtype (Liu et al., 1995, 1996; Quirk et al., 1996; Skolnick et al., 1997), it is tempting to generalise and hypothesise that, like L-655,708, RY-010, RY-023, RY-024 and RY-080 also have inverse agonist efficacy at the α1, α2 and α3 subtypes. Indeed, RY-080 has inverse agonism at the α1 subtype (Atack et al., 2006a).

L-655,708 had no effect following a priming stimulus (i.e., did not affect short-term potentiation; Seabrook et al., 1997), but following a burst stimulus it was able to enhance LTP, increasing the EPSP rise time from 291 ± 28% to 395 ± 48% of baseline (Fig. 3). Whilst it is possible that inverse agonism at the α5 subtype may be responsible for the enhancement in LTP observed with non-selective inverse agonists, such as DMCM, β-CCE and CGS-8216 (Yasui et al., 1993; Seabrook et al., 1997), it is important to note that under a stimulation paradigm similar that that used in the present study, DMCM was also able to enhance the short-term potentiation seen after a priming stimulus (Seabrook et al., 1997). This may indicate either that inverse agonism at other subtypes (i.e., α1-, α2- or α3-containing $GABA_A$ receptors) may also play a role in DMCM-mediated LTP or, alternatively, that the α5 inverse agonism of L-655,708, which is much less than that of DMCM (Fig. 2), is insufficient to enhance LTP to an extent that is comparable to DMCM. In this latter regard, the efficacy-selective compound α5IA (cf. L-655,708 which is binding selective; see Atack, 2005 for a more detailed description of affinity- versus efficacy-selectivity), has greater α5 inverse agonism than L-655,708 (−40% for α5IA versus −17% for L-655,708) and was able, like DMCM, to enhance the extent of LTP seen after a priming stimulus (Dawson et al., 2006).

The fact that L-655,708 enhances LTP in a mouse hippocampal slice model suggests that this compound can affect synaptic plasticity in a use-dependent manner (i.e., it does not affect baseline response rates) and may enhance cognition in vivo (Cooke and Bliss, 2005) in the same way that non-selective inverse agonists also enhance cognition (McNamara and Skelton, 1993). Since the expression of α5-containing $GABA_A$ receptors is highest in the hippocampus (Wisden et al., 1992; Pirker et al., 2000) and the hippocampus is associated with spatial learning (Eichenbaum et al., 1990), L-655,708 was evaluated in the Morris water maze (Morris, 1984). L-655,708 enhanced performance during the acquisition phase (“learning”) as well as in the probe trial (“memory”) in normal rats and is consistent with the performance enhancing effects of α5IA in the water maze (Dawson et al.,

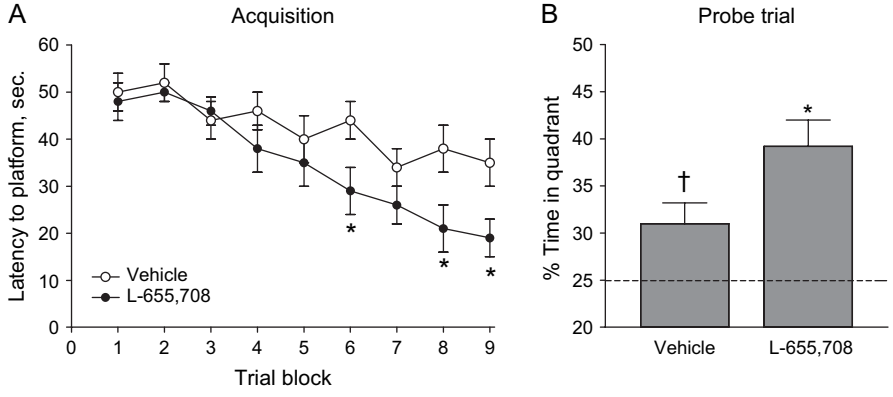

Fig. 6. L-655,708 enhances acquisition (“learning”) and probe trial (“memory”) performance in the Morris water maze. (A) The time taken to find the hidden platform decreased as a function of trial but the rate of this acquisition was greater in the L-655,708-treated animals. (B) Vehicle-treated rats spent significantly more time in the target quadrant than by chance alone (25%; †$p < 0.05$) but this memory of where the hidden platform had been located was significantly greater for L-655,708- relative to vehicle-treated rats (*$p < 0.05$). Values shown are mean ± SEM (n = 8/group).

2006). However, these studies with L-655,708 and α5IA are not directly comparable since due to the pellet formulation of L-655,708 the design of the water maze experiment used in the present study (i.e., the requirement to test each animal within a 1-day period) differed from the delayed-matching-to-position version of this assay used to study α5IA over a period of several days (Steele and Morris, 1999; Dawson et al., 2006). In the present study, the dose of L-655,708 that produced a cognition-enhancing effect corresponded to α5 occupancy of 75% whereas the minimum effective dose for α5IA occurred at 25% occupancy (Dawson et al., 2006). However, whilst it would be expected that due to its lower intrinsic α5 efficacy the α5 occupancy of L-655,708 required to enhance cognition would be greater than for α5IA, no minimum effective dose for L-655,708 was established and therefore it is not possible to relate α5 occupancy to the intrinsic efficacies of L-655,708 and α5IA.

The critical issue for the potential development of α5 selective inverse agonists as clinically useful cognition enhancers is whether or not inverse agonism at the α5 subtype is associated with proconvulsant activity. Thus, it has been previously reported that the α5 binding-selective compounds RY-023, RY-024 and RY-080 are all either convulsant or proconvulsant (Liu et al., 1996), implying that these effects are related to inverse agonist efficacy at the α5 subtype. In contrast, L-655,708 differentiated itself from these compounds, as well as the non-selective inverse agonists, in that it did not possess the proconvulsant activity. This discrepancy may be related to dose and/or pharmacokinetics since doses of RY-080 that are proconvulsant not only occupy α5-containing receptors but also give appreciable (albeit lower) occupancy at the α1, α2 and α3 subtypes (Atack et al., 2006a), at which RY-080 is presumed to also be an inverse agonist (see above). In the present study, the dose and formulation of L-655,708 was chosen such that fluctuations in the peak-to-trough plasma drug concentrations were minimized (Atack et al., 2006b) and the dose selected preferentially occupied α5-containing receptors. The importance of pharmacokinetics is demonstrated by the observation that L-655,708 was proconvulsant in mice when administered intraperitoneally as a bolus which occupies not only the α5 but also α1, α2 and α3 subtype (data not shown). The fact that α5-mediated cognitive enhancement can be achieved in the absence of proconvulsant activity is further highlighted by the efficacy-selective compound α5IA which was not only without proconvulsant activity but also did not produce kindling upon prolonged administration (Dawson et al., 2006).

Although L-655,708 has been reported to possess anxiogenic-like activities (Navarro et al., 2002, 2004) it is possible that these effects may not be mediated via the α5 subtype since α5-selective doses were not used in these studies. Moreover, deletion of the α5 subunit did not alter the behaviour of mice on the elevated plus maze (Collinson et al., 2002) nor did reduced expression of this subtype alter the anxiolytic efficacy of diazepam (Crestani et al., 2002). Finally, the α5 efficacy-selective compound α5IA was not anxiogenic (Dawson et al., 2006), all of which suggest that the α5 subtype is not related to anxiety.

Taken together, the results for L-655,708 support the hypothesis that α5-selective inverse agonists can enhance cognition without the proconvulsant and presumably anxiogenic activities. In addition, the fact that L-655,708 (and α5IA) enhanced performance in normal rats (cf., for example, the effects of the cholinesterase inhibitor Donepezil on cholinergically-compromised rats; Ogura et al., 2000) suggests that these compounds may have clinical utility in conditions, such as age-related cognitive decline, that are not generally associated with an underlying pathophysiology.

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