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Pharmacological properties of the $GABA_A$ receptor complex from brain regions of (hypoemotional) Roman high- and (hyperemotional) low-avoidance rats

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Abstract

The pharmacological properties of benzodiazepine binding sites of the γ-aminobutyric acid $(GABA)_A$ receptor complex from cortical, hippocampal and cerebellar membranes of Roman high-avoidance (RHA/Verh) and Roman low-avoidance (RLH/Verh) rats were investigated. No major differences between the two lines were found in the binding parameters of [^{3}H]flunitrazepam (a non-selective agonist), [^{3}H]zolpidem (a Type I selective agonist) or [^{3}H]ethyl 8-azido-6-dihydro-5-methyl-6-oxo-4*H*-imidazol[1,5-*a*]-[1,4]benzodiazepine-3-carboxylate (Ro15-4513) (a partial inverse agonist). Neither the K_d values nor the B_{max} for these ligands differed between RHA/Verh and RLA/Verh rats in any of the brain regions studied. As a result, the proportion of Type I binding sites in cortical and hippocampal membranes of RHA/Verh and RLA/Verh rats or the 'diazepam-sensitive' and the 'diazepam-insensitive' binding sites in cerebellar membranes, calculated from the [^{3}H]flunitrazepam and [^{3}H]zolpidem maximal binding sites or from [^{3}H]Ro15-4513 binding (in the absence or in presence of diazepam), respectively, was also similar. Furthermore, there were no differences between the two rat lines in the allosteric interactions between GABA and the benzodiazepine binding sites (labeled with [^{3}H]flunitrazepam) in all three areas tested or the Type I binding sites (labeled with [^{3}H]zolpidem) in the hippocampus. In contrast, RLA/Verh rats showed a significant reduction in the allosteric interactions between GABA and [^{3}H]zolpidem binding sites in the cortex. As a whole, these results indicate the absence of generalized between-line differences in the $GABA_A$ receptor complex showing, at the same time, the existence of some specific differences in allosterism within the $GABA_A$ complex. These differences may contribute to the divergent emotional responses which characterize the RHA/Verh and RLA/Verh rat lines.

Keywords: $GABA_A$ receptor complex; [^{3}H]Flunitrazepam; [^{3}H]Zolpidem; Diazepam; [^{3}H]Ro15-4513; Cortex; Hippocampus; Cerebellum; Emotionality; (RHA/Verh rats); (RLA/Verh rats)

1. Introduction

The Wistar-derived Roman high- and low-avoidance (RHA/Verh and RLA/Verh, respectively) rat lines have been selected and bred in Switzerland since 1972 for rapid acquisition (RHA/Verh) vs. extremely poor acquisition (RLA/Verh) of two-way active (shuttle box) avoidance (Driscoll and Bättig, 1982), using stock previously developed by Bignami and Broadhurst (Bignami, 1965; Broadhurst and Bignami, 1965).

In accordance with the evidence showing that early, two-way active avoidance acquisition is mediated by fear-motivated responses (Fernández-Teruel et al., 1991a), extensive research conducted with the Roman/Verh rats has led to the conclusion that the RLA/Verh line is more emotionally reactive than the RHA/Verh line. For example, RLA/Verh rats show, as compared to RHA/Verh animals, a more pronounced tachycardia when exposed to various unconditioned stressful conditions, a more intense

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bradycardia in response to a conditioned emotional stressor, and increased stressor- or novelty-induced ACTH, corticosterone, prolactin, self-grooming, defecation and freezing responses (Driscoll et al., 1998 and Escorihuela et al., 1995, for recent reviews). Likewise, RLA/Verh rats display stronger conflict-induced suppression of drinking and hyponeophagia than do RHA/Verh rats (Ferré et al., 1995), thus giving more definitive support to the contention that the RLA/Verh line is the more anxious of the two.

It is well established that, among others, the GABAergic system plays an important role in anxious/emotional behavior and stress responses (e.g., Biggio et al., 1990; Fernández-Teruel et al., 1991b; Trullás et al., 1988). In this connection, it is known that RHA/Verh and RLA/Verh rats show divergences in their behavioral and neurochemical sensitivity to the effects of drugs acting at the $GABA_A$ receptor complex, such as chlordiazepoxide, diazepam, pentobarbital, flumazenil, pentylenetetrazol and ethanol (Corda et al., 1997; Driscoll et al., 1995; Driscoll and Stübi, 1985; Escorihuela et al., 1995; Fernández-Teruel et al., 1991b; Martin et al., 1982).

At the neurochemical level, in addition to differences which have been noted between the lines in other transmitter systems (e.g., Driscoll et al., 1983, 1990), recent studies have shown that RLA/Verh rats display less cortical GABA-stimulated chloride uptake (Giorgi et al., 1994) and lower levels of cortical progesterone metabolites (which interact with the $GABA_A$ receptor and have anxiolytic properties; Steimer et al., 1997) than do their RHA/Verh counterparts. As it remained to be established whether there are also differences in the pharmacological properties of $GABA_A$ receptor subtypes, the present work aimed at studying these pharmacological characteristics, as well as the allosteric interactions of benzodiazepine binding sites, in the brains of RHA/Verh and RLA/Verh rats.

2. Materials and methods

2.1. Animals

Naive, male RHA/Verh and RLA/Verh rats (approximately 6 months old) were used for the experiments. All experiments were carried out at the Department of Biochemistry, Bromatology and Toxicology, School of Pharmacy, University of Sevilla, Spain.

2.2. Materials

[^{3}H]Flunitrazepam (86.6 Ci/mmol), [^{3}H]zolpidem (58.3 Ci/mmol) and [^{3}H]Ro15-4513 (24.1 Ci/mmol) were purchased from New England Nuclear. Benzodiazepines were kindly provided by Hofmann-LaRoche (USA) and GABA by Sigma (Madrid, Spain).

2.3. Membrane preparations

Following decapitation, cortex, hippocampus and cerebellum were dissected, frozen in liquid nitrogen and stored at −80°C until use.

The membranes were prepared according to Mernoff et al. (1983), as described in detail elsewhere (Ruano et al., 1991). Briefly, the different brain regions were homogenized in 0.32 M sucrose, 50 mM Tris–HCl (pH 7.4), 1 mM EGTA, 1 mM EDTA, 1 mM dithiotreitol and centrifuged at 130 000 × *g* for 30 min at 4°C. The pellets were resuspended without sucrose and frozen at −80°C for 18 h. The frozen membranes were thawed and washed four times in the same medium. Finally, the pellets were frozen at −80°C for 1 h, thawed and washed once. The membranes were finally resuspended in 50 mM Tris–HCl, pH 7.4, at a protein concentration of 3–4 mg/ml. These membrane preparations are virtually free of endogenous GABA (Ruano et al., 1993a).

2.4. Binding assays

For all binding assays, 0.2–0.4 mg of membrane protein was incubated in 0.2 ml (for saturation experiments) or in 0.4 ml (GABA enhancement experiments) of 50 mM Tris–HCl (pH 7.4) at 4°C for 45 min. Non-specific binding was determined in the presence of 5 μM diazepam or 5 μM Ro15-4513 for [^{3}H]flunitrazepam or [^{3}H]zolpidem and [^{3}H]Ro15-4513 binding, respectively. The binding was terminated by rapid filtration through Whatman GF/B glass-fiber filters.

For saturation experiments, the concentration of [^{3}H]ligands varied from 1 nM to 30 nM for [^{3}H]flunitrazepam and [^{3}H]Ro15-4513 (in the absence or presence of 5 μM diazepam) or from 1 nM to 10 nM for [^{3}H]zolpidem (Ruano et al., 1994; Gutierrez et al., 1997).

The allosteric interactions between GABA and benzodiazepine binding sites were tested by determining the effect of a single saturating concentration of GABA (100 μM) on the binding of 1 nM [^{3}H]flunitrazepam and 3 or 5 nM [^{3}H]zolpidem. The dose–response curves were obtained by determining the effect of eight different concentrations of GABA (ranging from 0.001 to 100 μM) on the binding of 5 nM [^{3}H]zolpidem.

The protein concentration was determined according to Lowry et al. (1951) using bovine serum albumin as standard.

2.5. Data analysis

The Scatchard transformation of the saturation curves was adjusted using LIGAND (Munson and Rodbard, 1980), and the dose–response curves for GABA enhancement of [^{3}H]zolpidem binding were fit as previously described (Ruano et al., 1991). The comparison between the means

of different groups of data was evaluated by analysis of variance (ANOVA) and Tukey tests.

3. Results

3.1. Pharmacological characterization of the benzodiazepine binding sites from cortical, hippocampal and cerebellar membranes of RHA / Verh and RLA / Verh rats

The pharmacological properties of the benzodiazepine binding sites from cortex, hippocampus and cerebellum were determined in saturation experiments using different ^{3}H-ligands. The total benzodiazepine binding sites, in the three brain areas tested, were determined using [^{3}H]flunitrazepam (a non-selective benzodiazepine agonist). In cortex and hippocampus, the Type I binding sites were quantified using [^{3}H]zolpidem. [^{3}H]Zolpidem, at low concentrations, binds specifically to the Type I benzodiazepine receptors (Ruano et al., 1994). We also determined the diazepam-sensitive and the diazepam-insensitive binding sites in cerebellar membranes, using [^{3}H]Ro15-4513 in the presence or absence of 5 μM diazepam (Turner et al., 1991; Gutierrez et al., 1997).

The Scatchard transformation of the saturation curves are shown in Tables 1 and 2. As expected, all three ligands bound to single, high-affinity binding sites in all three brain areas from both rat lines (Hill factors close to unity, data not shown). No line-dependent differences were observed in either the affinity (K_d) or the B_{max} for either binding site, i.e., [^{3}H]flunitrazepam, [^{3}H]zolpidem or [^{3}H]Ro15-4513, in cortex, hippocampus or cerebellum.

As shown in Table 1, the proportion of cortical and hippocampal Type I benzodiazepine binding sites, calculated from the B_{max} of [^{3}H]zolpidem and [^{3}H]flunitrazepam binding sites, was similar in both the RHA/Verh and RLA/Verh rats. Interestingly, the binding parameters (K_d, B_{max} and the proportion of Type I binding sites) of the benzodiazepine binding sites in cortical and hippocampal membranes from both rat lines were very similar to those observed in cortical and hippocampal membranes from Wistar or Fischer 344 rats (Ruano et al., 1993b, 1995). Furthermore, the binding parameters of the benzodiazepine binding sites from RHA/Verh or RLA/Verh cerebellar membranes (K_d, B_{max} and proportion of diazepam-sensitive and diazepam-insensitive binding sites) (see Table 2) were also comparable to those found in the Wistar rats (Gutierrez et al., 1997).

Therefore, these results indicate that the benzodiazepine binding sites in cortex, hippocampus and cerebellum are similar in both RHA/Verh and RLA/Verh rats and analogous to those of our Wistar or Fischer 344 rats.

3.2. Allosteric interactions between GABA and benzodiazepine binding sites from RHA / Verh or RLA / Verh cortical, cerebellar and hippocampal membranes

The allosteric interactions between GABA and benzodiazepine binding sites were first tested by determining the effect of a single saturating concentration of GABA (100 μM) on [^{3}H]flunitrazepam or [^{3}H]zolpidem binding. The results are shown in Fig. 1. As shown, in both RHA/Verh and RLA/Verh rats, the GABA enhancement of [^{3}H]flunitrazepam binding displayed regional heterogeneity. The stimulatory effect of GABA was higher in cortical and cerebellar membranes than in hippocampal membranes. These regional differences in the allosteric interactions between GABA and benzodiazepine binding sites were also observed in Wistar rats (Unnerstall et al., 1981; Santi et al., 1988; Corda et al., 1989; Ruano et al., 1993a). No line-dependent differences were observed in cortical, cerebellar or hippocampal membranes with this ligand (Fig. 1). There was a small (15–20%) decrease in the GABA enhancement of [^{3}H]flunitrazepam binding in RLA/Verh cortical membranes although this was not statistically significant.

We also tested the allosteric interactions between GABA and the Type I ([^{3}H]zolpidem binding) benzodiazepine binding sites from RHA/Verh and RLA/Verh cortical and hippocampal membranes. As shown in Fig. 1, in

Table 1
Benzodiazepine binding parameters of cortical and hippocampal membranes from RHA/Verh and RLA/Verh rats

Brain area	Rat line	Binding parameters			
		[^{3}H]flunitrazepam		[^{3}H]zolpidem	
		K_d (nM)	B_{max} (pmol/mg protein)	K_d (nM)	B_{max} (pmol/mg protein)
Cortex	RHA/Verth	1.8 ± 1.4	1.12 ± 0.30	7.0 ± 1.1	0.54 ± 0.10 (48.2 ± 5.7%)
	RLA/Verth	2.6 ± 0.6	1.10 ± 0.30	7.2 ± 5.2	0.57 ± 0.11 (52.3 ± 9.8%)
Hippocampus	RHA/Verth	1.3 ± 0.4	1.20 ± 0.20	8.0 ± 3.9	0.43 ± 0.09 (36.3 ± 6.6%)
	RLA/Verth	1.2 ± 0.2	1.00 ± 0.20	7.0 ± 0.1	0.42 ± 0.10 (42.5 ± 9.5%)

Values were calculated from [^{3}H]flunitrazepam or [^{3}H]zolpidem saturation curves by LIGAND.
The results are means ± S.D. of five to six experiments performed in duplicate.
One animal from each line was used in each experiment (n = 5–6/rat line).
The proportion of Type I binding sites is given in parentheses.

Table 2
Binding parameters of the benzodiazepine binding sites of cerebellar membranes from RHA/Verh and RLA/Verh rats

Rat line	Binding parameters							
	[^{3}H]FNZ		[^{3}H]Ro15-4513					
			Total		DS		DI	
	K_d	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d	B_{max}
RHA/Verh	1.8 ± 0.6	0.54 ± 0.17	4.0 ± 3.6	2.12 ± 0.18	4.3 ± 2.8	1.40 ± 0.21 (66.0 ± 9.9%)	4.2 ± 4.5	0.71 ± 0.12 (33.5 ± 5.6%)
RLA/Verh	2.2 ± 0.5	0.63 ± 0.10	5.8 ± 1.6	1.8 ± 0.24	5.3 ± 2.5	1.16 ± 0.18 (63.0 ± 9.7%)	3.9 ± 4.2	0.62 ± 0.12 (33.7 ± 6.5%)

Data were calculated from saturation experiments by LIGAND and are means ± S.D. of six experiments performed in duplicate ($n = 6$ animals/rat line). The total and the diazepam-insensitive (DI) [^{3}H]Ro15-4513 binding parameters were determined in the absence or presence of 5 μM diazepam, respectively.
Diazepam-sensitive binding was calculated from total and DI binding.
The K_d and the B_{max} are expressed in nM and pmol/mg of proteins, respectively.
The proportion of DS and DI binding sites is given in parentheses.

RHA/Verh rats the stimulatory effect of GABA on [^{3}H]zolpidem binding was 1.5–2 times higher than that observed with [^{3}H]flunitrazepam. The highest stimulatory effect of GABA was observed in cortical membranes (Fig. 1). These results are in close agreement with those reported previously for cortical and hippocampal membranes from Wistar or Fischer 344 rats (Ruano et al., 1993a,b, 1995). The effect of GABA on [^{3}H]zolpidem binding in RLA/Verh cortical membranes was significantly lower than that in RHA/Verh cortical membranes (see Fig. 1). Furthermore, in RLA/Verh cortical membranes, GABA stimulation of [^{3}H]zolpidem binding was similar to that of [^{3}H]flunitrazepam binding and slightly lower than that found in RLA/Verh hippocampal membranes.

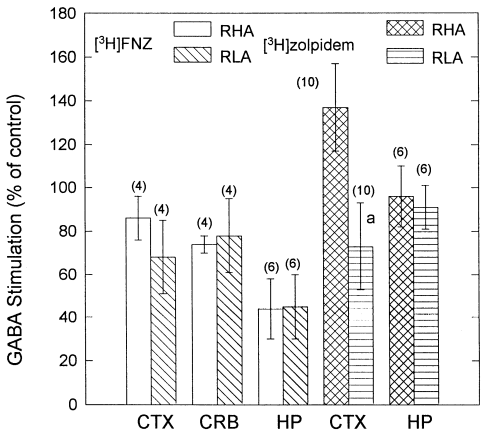

Fig. 1. GABA stimulation of [^{3}H]flunitrazepam (open and hatched bars) or [^{3}H]zolpidem (cross-hatched and horizontal line bars) binding sites in cortical (CTX), cerebellar (CRB) and hippocampal (HP) membranes from RHA/Verh (open and cross-hatched bars) or RLA/Verh (hatched and horizontal line bars) rats. The membranes were incubated in the presence of 100 μM GABA and 1 nM [^{3}H]flunitrazepam or 3 nM [^{3}H]zolpidem. The data, expressed as percentage of stimulation, are means ± S.D. of experiments performed in duplicate. One animal from each rat line was used for each experiment. Number of animals/group is given in parentheses. [a]Significant difference between RHA/Verh (RHA) and RLA/Verh (RLA) rats, ANOVA $F_{1,18} = 31.45$, $p < 0.0001$; Tukey $p < 0.01$.

The decrease in GABA enhancement of [^{3}H]zolpidem binding, observed in the cortex of RLA/Verh rats, could be due to modification of either the potency (EC_{50}) and/or the efficacy (E_{max}) of GABA. Thus, in order to ascertain which of these two parameters was modified in RLA/Verh rats, dose–response curves were generated. The results of these experiments and the fit of the data are shown in Fig. 2 and Table 3, respectively. As expected, in both RHA/Verh and RLA/Verh cortical membranes, GABA enhanced [^{3}H]zolpidem binding in a dose-dependent manner. In RLA/Verh rats, neither the potency nor the Hill factor was altered, as compared to the RHA/Verh values (Table 3). However, the efficacy (E_{max}) of GABA in enhancing [^{3}H]zolpidem binding decreased significantly in cortical membranes from RLA/Verh rats (Fig. 2 and Table 3). Thus, these results indicate that the decrease in

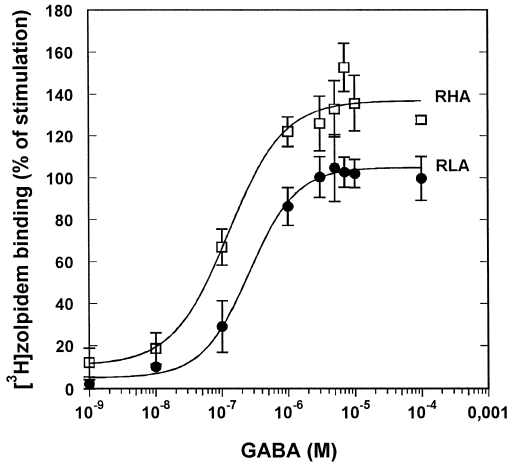

Fig. 2. Allosteric interaction between GABA and [^{3}H]zolpidem binding to Type I benzodiazepine receptors. The allosteric enhancement of 5 nM [^{3}H]zolpidem binding was determined in cortical membranes from RHA/Verh (open symbols) or RLA/Verh (closed symbols) rats. The data, i.e., the mean ± S.D. of three different animals of each line, are expressed as percentages of stimulation of [^{3}H]zolpidem binding in the absence of GABA.

Table 3
Enhancement by GABA of [^{3}H]zolpidem binding in hippocampal membranes from RHA/Verh or RLA/Verh rats

Rat line	GABA stimulation of [^{3}H]zolpidem binding		
	E_{max} (% of control)	EC_{50} (μM)	n_H
RHA/Verh	136 ± 17	0.4 ± 0.3	1.3 ± 0.5
RLA/Verh	95 ± 16[a]	0.5 ± 0.3	1.4 ± 0.7

[a] Significant difference, ANOVA $F_{1,5} = 12.6$, Tukey $P < 0.05$.
The maximal stimulation (E_{max}), the potency (EC_{50}) and the Hill slope (n_H) were calculated from the dose–response curves shown in Fig. 2. Values are the means ± S.D. of three experiments performed in duplicate ($n = 3$ animals/rat line).

the GABA enhancement of [^{3}H]zolpidem binding in RLA/Verh cortical membranes was due to a decrease in the efficacy of the allosteric interactions between the GABA and the Type I binding sites.

4. Discussion

The results of the present study indicated an absence of generalized differences in the pharmacological properties of the total benzodiazepine binding sites from cortex, hippocampus or cerebellum of the RHA/Verh and RLA/Verh rat lines.

The existence of genetic differences in the pharmacological properties of the $GABA_A$ receptor complex has been investigated with different [^{3}H]-ligands but controversial results have been reported (Gentsch et al., 1981; Shephard et al., 1982; Giorgi et al., 1994; Driscoll et al., 1998). Our results, in agreement with those reported by Giorgi et al. (1994) and Corda et al. (1997), demonstrated that both the affinity and the maximal density of [^{3}H]flunitrazepam binding sites were identical in both lines. However, the benzodiazepine binding sites are heterogeneous. Classically, two pharmacologically different benzodiazepine binding isotypes have been described (see Sieghart, 1995; Hadingham et al., 1996; Luddens et al., 1990; Sieghart, 1995). Thus, the absence of differences in the total density (B_{max}) of the benzodiazepine binding sites between RHA/Verh and RLA/Verh rats does not allow us to rule out the possibility of differential redistribution of the different benzodiazepine binding subtypes. Therefore, we also determined the binding properties of the Type I binding sites (high affinity for [^{3}H]zolpidem) in the cortex and hippocampus and the diazepam-sensitive and diazepam-insensitive binding sites in the cerebellum. As shown in Tables 1 and 2, no line-dependent differences were observed in such measures. These results indicated the absence of line-dependent differences in the distribution of the different benzodiazepine binding sites between RHA/Verh and RLA/Verh rats.

The different benzodiazepine binding sites reflect the presence of different α subunits in the $GABA_A$ receptor complex (Hadingham et al., 1996; Pritchett and Seeburg, 1990; Pritchett et al., 1989). Type I binding sites are determined by the presence of $\alpha 1$ subunits, Type II by the presence of $\alpha 2$, 3 or 5 subunits, and diazepam-insensitive Ro15-4513 binding sites by the presence of $\alpha 4$ or 6 subunits. The α1-2-3-5 subunits are expressed in cortex and hippocampus, whereas only $\alpha 1$ and $\alpha 6$ subunits are significantly expressed in cerebellum (all other α subunits are minor components of the $GABA_A$ receptor in this particular brain region) (Laurie et al., 1992; Wisden et al., 1992). Therefore, the maximal density of [^{3}H]zolpidem and diazepam-sensitive [^{3}H]Ro15-4513 binding sites or diazepam-insensitive [^{3}H]Ro15-4513 binding sites should reflect the presence of $\alpha 1$ and $\alpha 6$ subunits, respectively, in functional $GABA_A$ receptor complexes. In consequence, the absence of line-dependent divergences in the total benzodiazepine binding sites, [^{3}H]zolpidem binding in cortex and hippocampus, and diazepam-sensitive or diazepam-insensitive [^{3}H]Ro15-4513 binding sites in cerebellum indicate a similar expression of $\alpha 1$ and $\alpha 6$ subunits between both rat lines and suggest the absence of differences in the relative abundance of the other α subunits of the $GABA_A$ receptor complex.

We also compared the allosteric interactions between GABA and both the total benzodiazepine and the Type I binding sites in the cortex, hippocampus and cerebellum of RHA/Verh and RLA/Verh rats. As shown (see Fig. 1), no line-dependent differences were observed in the cerebellum and hippocampus. Nevertheless, the GABA effect on the Type I binding sites was significantly reduced in cortical membranes from RLA/Verh rats. A parallel reduction was also observed in the total benzodiazepine binding sites, though this difference did not reach statistical significance, indicating an absence of modification of the allosteric interactions between GABA and the benzodiazepine binding sites distinct than the Type I (high affinity for zolpidem).

The allosteric interactions between GABA binding sites and either total benzodiazepine binding sites or the Type I binding sites show regional heterogeneity in Wistar or Fischer 344 rats (Unnerstall et al., 1981; Santi et al., 1988; Corda et al., 1989; Ruano et al., 1993a). The GABA enhancement of [^{3}H]flunitrazepam binding was maximal in the cortex, followed by cerebellum and hippocampus. A similar profile could be observed for the Type I binding sites, although the GABA effect on [^{3}H]zolpidem binding was higher than that for [^{3}H]flunitrazepam. An identical pattern was observed here in RHA/Verh rats (Fig. 1), suggesting that the difference between RHA/Verh and RLA/Verh rats found in the cortex is due to a reduction of the GABA stimulation of [^{3}H]zolpidem binding in RLA/Verh rats, rather than to an increase in RHA/Verh rats. Interestingly, the existence of genetic differences in the allosteric coupling between the GABA site and the Cl-channel between both rat lines were recently reported (Giorgi et al., 1994), with RLA/Verh rats showing a

reduction of GABA-stimulated Cl^- uptake. Therefore, the differences in the allosteric interactions between GABA and [^{3}H]zolpidem binding sites could be reflected in the Cl^- uptake properties of the $GABA_A$ receptor complex. Taken together, these results suggest the absence of between-line differences in the maximal density of binding sites, and the existence of line-dependent divergences in the allosteric coupling among the different binding sites and the Cl^- channel of the $GABA_A$ receptor complex at the cortical level.

It is known that the allosteric properties of the $GABA_A$ receptor are influenced by steroid hormones (Olsen et al., 1988). Related to this, it has been recently reported that the brain content of anxiolytic progesterone metabolites is higher in the frontal cortex and bed nucleus of the stria terminalis in RHA/Verh than in RLA/Verh rats (Steimer et al., 1997). Interestingly, the levels of these neuroactive steroid metabolites have been shown to be negatively correlated to measures of emotional reactivity in both Roman/Verh rat lines. Thus, it is tempting to speculate that the differences observed in the allosteric coupling between cortical Type I and GABA binding sites might be related to the differences in emotional reactivity (or susceptibility to stress) and/or in neurosteroid activity seen between the Roman/Verh rat lines.

Alternatively, we cannot discard the possibility that there are molecular and/or post-translational modifications of the $GABA_A$ receptor complex in RHA/Verh and RLA/Verh rats. In this respect, it is known that the presence of two different α subunits (such as $\alpha 1$ and $\alpha 3$) co-assembled in a single $GABA_A$ receptor complex display unique allosteric properties different from those of $\alpha 1$ or $\alpha 3$ receptors (Ebert et al., 1994). Moreover, the existence of native $GABA_A$ receptors containing two different α subunits (such as $\alpha 1$–$\alpha 3$, $\alpha 1$–$\alpha 2$ and $\alpha 1$–$\alpha 6$ subunits) has been demonstrated in immunopurification experiments (Araujo et al., 1996; Duggan et al., 1991; McKernan et al., 1991; Pollard et al., 1993, 1995). Therefore, the observed between-line differences in the allosteric interactions could also reflect molecular differences in the composition of the $GABA_A$ receptor complex.

In summary, our results demonstrate the absence of major differences between RHA/Verh and RLA/Verh rats in benzodiazepine binding properties (affinity, B_{max}, relative abundance of Type 1 and diazepam-sensitive or diazepam-sensitive bindings sites) and in the allosteric interactions between benzodiazepine and GABA binding sites in the cortex, hippocampus or cerebellum. Our results also demonstrate the existence of a significant reduction in the allosteric interactions between [^{3}H]zolpidem (a Type I-selective agonist) and GABA binding sites in cortical membranes from the RLA/Verh rat line. This decrease in the allosteric interactions of the Type I subtype of the $GABA_A$ receptor complex could contribute to the divergent emotional responses which characterize these rats.

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References

Araujo, F., Tan, S., Ruano, D., Schoemaker, H., Benavides, J., Vitorica, J., 1996. Molecular and pharmacological characterization of native cortical gamma-aminobutyric $acid_A$ receptors containing both alpha 1 and alpha 3 subunits. J. Biol. Chem. 271, 27902–27911.

Biggio, G., Concas, A., Corda, M.G., Giorgi, O., Sanna, E., Serra, M., 1990. GABAergic and dopaminergic transmission in the rat cerebral cortex: effect of stress, anxiolytic and anxiogenic drugs. Pharmacol. Ther. 48, 121–142.

Bignami, G., 1965. Selection for high rates and low rates of avoidance conditioning in the rat. Anim. Behav. 13, 221–227.

Broadhurst, P.L., Bignami, G., 1965. Correlative effects of psychogenetic selection: a study of the Roman high- and low-avoidance strains of rats. Behav. Res. Ther. 2, 273–280.

Corda, M.G., Giorgi, O., Longoni, B., Ongini, E., Pesce, G., Cruciani, R., Biggio, G., 1989. Functional coupling of $GABA_A$ receptors and benzodiazepine recognition site subtypes in the spinal cord of the rat. Eur. J. Pharmacol. 169, 205–213.

Corda, M.G., Lecca, D., Piras, G., Di Chiara, G., Giorgi, O., 1997. Biochemical parameters of dopaminergic and GABAergic neurotransmission in the CNS of Roman high-avoidance and Roman low-avoidance rats. Behav. Genet. 27, 527–536.

Driscoll, P., Bättig, K., 1982. Behavioral, emotional and neurochemical profiles of rats selected for extreme differences in active, two-way avoidance performance. In: Lieblich, I. (Ed.), Genetics of the Brain. Elsevier, Amsterdam, pp. 95–123.

Driscoll, P., Stübi, R., 1985. Dose-related effects of pentobarbital on the genetic differences seen between paired, Roman high- or low-avoidance rats in a shuttle box. Pharmacol. Biochem. Behav. 22, 435–439.

Driscoll, P., Dedek, J., Martin, J.R., Zivkovic, B., 1983. Two-way avoidance and acute shock stress induced alterations of regional noradrenergic, dopaminergic and serotonergic activity in Roman high- and low-avoidance rats. Life Sci. 33, 1719–1725.

Driscoll, P., Dedek, J., D'Angio, M., Claustre, Y., Scatton, B., 1990. A genetically-based model for divergent stress responses: behavioral, neurochemical and hormonal aspects. Adv. Anim. Breeding Genet. 5, 97–107.

Driscoll, P., Ferré, P., Fernández-Teruel, A., Levi de Stein, M., Wolfman, C., Medina, J., Tobeña, A., Escorihuela, R.M., 1995. Effects of prenatal diazepam on two-way avoidance behavior, swimming navigation and brain levels of benzodiazepine-like molecules in male Roman high- and low-avoidance rats. Psychopharmacology 122, 51–57.

Driscoll, P., Escorihuela, R.M., Fernández-Teruel, A., Giorgi, O., Schwegler, H., Steimer, Th., Wiersma, A., Corda, M.G., Flint, J., Koolhaas, J., Langhans, W., Schulz, P.E., Siegel J., Tobeña, A., 1998. Genetic selection and differential stress responses: the Roman lines/strains of rats. Ann. N.Y. Acad. Sci., in press.

Duggan, M.J., Pollard, S., Stephenson, F.A., 1991. Immunoaffinity purification of $GABA_A$ receptor α subunit iso-oligomers. J. Biol. Chem. 266, 24778–24784.

Ebert, B., Wafford, K.A., Whiting, P.J., Krogsgaardlarsen, P., Kemp, J.A., 1994. Molecular pharmacology of gamma-aminobutyric acid type A receptor agonists and partial agonists in oocytes injected with different alpha, beta, and gamma receptor subunit combinations. Mol. Pharmacol. 46, 957–963.

Escorihuela, R.M., Tobeña, A., Driscoll, P., Fernández-Teruel, A., 1995.

Effects of training, early handling, and perinatal flumazenil on shuttle box acquisition in Roman low-avoidance rats: toward overcoming a genetic deficit. Neurosci. Biobehav. Rev. 19, 353–367.

Fernández-Teruel, A., Escorihuela, R.M., Núñez, J.F., Zapata, A., Boix, F., Salazar, W., Tobeña, A., 1991a. The early acquisition of two-way (shuttle box) avoidance as an anxiety-mediated behavior: psychopharmacological validation. Brain Res. Bull. 26, 173–176.

Fernández-Teruel, A., Escorihuela, R.M., Tobeña, A., Driscoll, P., 1991b. Stress and putative endogenous ligands for benzodiazepine receptors: the importance of characteristics of the aversive situation and of differential emotionality of experimental animals. Experientia 47, 1051–1056.

Ferré, P., Fernández-Teruel, A., Escorihuela, R.M., Driscoll, P., Corda, M.G., Giorgi, O., Tobeña, A., 1995. Behavior of the Roman/Verh high- and low-avoidance rat lines in anxiety tests: relationship with defecation and self-grooming. Physiol. Behav. 58, 1209–1231.

Gentsch, C., Lichtsteiner, M., Feer, H., 1981. 3H-diazepam binding sites in Roman high- and low-avoidance rats. Experientia 37, 1315–1316.

Giorgi, O., Orlandi, M., Escorihuela, R.M., Driscoll, P., Lecca, D., Corda, M.G., 1994. GABAergic and dopaminergic transmission in the brain of Roman high-avoidance and Roman low-avoidance rats. Brain Res. 638, 133–138.

Gutierrez, A., Khan, Z.U., Miralles, C.P., Mehta, A.K., Ruano, D., Araujo, F., Vitorica, J., deBlas, A.L., 1997. $GABA_A$ receptor subunit expression changes in the rat cerebellum and cerebral cortex during aging. Mol. Brain Res. 45, 59–70.

Hadingham, K.L., Garrett, E.M., Wafford, K.A., Bain, C., Heavens, R.P., Sirinathsinghji, D.J.S., Whiting, P.J., 1996. Cloning of cDNAs encoding the human gamma-aminobutyric acid type A receptor alpha 6 subunit and characterization of the pharmacology of alpha 6-containing receptors. Mol. Pharmacol. 49, 253–259.

Laurie, D.J., Seeburg, P.H., Wisden, W., 1992. The distribution of l13 $GABA_A$ receptor subunit mRNAs in the rat brain: II. Olfactory bulb and cerebellum. J. Neurosci. 12, 1063–1076.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Luddens, H., Pritchett, D.B., Kohler, M., Killisch, I., Keinanen, K., Monyer, H., Sprengel, R., Seeburg, P.H., 1990. Cerebellar $GABA_A$ receptor selective for a behavioral alcohol antagonist. Nature 346, 648–651.

Martin, J.R., Oettinger, R., Driscoll, P., Buzzi, R., Bättig, K., 1982. Effects of chlordiazepoxide and imipramine on maze patrolling within two different maze configurations by psychogenetically selected lines of rats. Psychopharmacology 78, 58–62.

McKernan, R.M., Quirk, K., Prince, R., Cox, P.A., Gillard, N.P., Ragan, C.I., Whiting, P., 1991. $GABA_A$ receptor subtypes immunopurified from rat brain with α subunit-specific antibodies have unique pharmacological properties. Neuron 7, 667–676.

Mernoff, S.T., Cherwinski, H.M., Becker, J.W., De Blas, A.L., 1983. Solubilization of brain benzodiazepine receptor with a zwitterionic detergent: optimal preservation of their functional interaction with GABA receptor. J. Neurochem. 41, 752–758.

Munson, P.J., Rodbard, D., 1980. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 107, 220–239.

Olsen, R.W., Yang, J.S., Ransom, R.W., 1988. GABA-stimulated 36Cl-flux in brain slices as an assay for modulation by CNS depressant drugs. Adv. Biochem. Psychopharmacol. 45, 125–133.

Pollard, S., Duggan, M.J., Stephenson, F.A., 1993. Further evidence for the existence of α subunit heterogeneity within discrete gamma-aminobutyric acid(A) receptor subpopulations. J. Biol. Chem. 268, 3753–3757.

Pollard, S., Thompson, C.L., Stephenson, F.A., 1995. Quantitative characterization of alpha 6 and alpha 1 alpha 6 subunit-containing native gamma-aminobutyric acid(A) receptors of adult rat cerebellum demonstrates two alpha subunits per receptor oligomer. J. Biol. Chem. 270, 21285–21290.

Pritchett, D.B., Seeburg, P.H., 1990. Gamma-aminobutyric acid(A) receptor alpha 5 subunit creates novel type II benzodiazepine receptor pharmacology. J. Neurochem. 54, 1802–1804.

Pritchett, D.B., Luddens, H., Seeburg, P.H., 1989. Type I and type II $GABA_A$-benzodiazepine receptors produced in transfected cells. Science 245, 1389–1392.

Ruano, D., Cano, J., Machado, A., Vitorica, J., 1991. Pharmacologic characterization of $GABA_A$/benzodiazepine receptor in rat hippocampus during aging. J. Pharmacol. Exp. Ther. 256, 902–908.

Ruano, D., Benavides, J., Machado, A., Vitorica, J., 1993a. Regional differences in the enhancement by GABA of [^{3}H]zolpidem binding to ω_1 sites in rat brain membranes and sections. Brain Res. 600, 134–140.

Ruano, D., Machado, A., Vitorica, J., 1993b. Absence of modifications of the pharmacological properties of the $GABA_A$ receptor complex during aging, as assessed in 3- and 24-month-old rat cerebral cortex. Eur. J. Pharmacol. Mol. Pharmacol. 246, 81–87.

Ruano, D., Araujo, F., Machado, A., De Blas, A.L., Vitorica, J., 1994. Molecular characterization of type I $GABA_A$ receptor complex from rat cerebral cortex and hippocampus. Mol. Brain Res. 25, 225–233.

Ruano, D., Benavides, J., Machado, A., Vitorica, J., 1995. Aging-associated changes in the pharmacological properties of the benzodiazepine (w) receptor isotypes in the rat hippocampus. J. Neurochem. 64, 867–873.

Santi, M.R., Cox, D.H., Guidotti, A., 1988. Heterogeneity of γ-aminobutyric acid/benzodiazepine/β-carboline receptor complex in rat spinal cord. J. Neurochem. 50, 1080–1086.

Shephard, R.A., Nielsen, E.B., Broadhurst, P.L., 1982. Sex and strain differences in benzodiazepine receptor binding in Roman rat strains. Eur. J. Pharmacol. 77, 327–330.

Sieghart, W., 1995. Structure and pharmacology of gamma-aminobutyric acid(A) receptor subtypes. Pharmacol. Rev. 47, 181–234.

Steimer, Th., Driscoll, P., Schulz, P., 1997. Brain metabolism of progesterone, coping behavior and emotional reactivity in male rats from two psychogenetically selected lines. J. Neuroendocrinol. 9, 169–175.

Trullás, R., McIntyre, T., Skolnick, P., 1988. The benzodiazepine GABA chloride ionophore complex as a biowarning system. In: Tagaki, H., Oomura, Y., Ito, M., Otsuka M. (Eds.), Biowarning System in the Brain, Biowarning System in the Brain. Univ. Tokyo Press, Tokyo, pp. 227–241.

Turner, D.M., Sapp, D.W., Olsen, R.W., 1991. The benzodiazepine/alcohol antagonist Ro15-4513: binding to a $GABA_A$ receptor subtype that is insensitive to diazepam. J. Pharmacol. Exp. Ther. 257, 1236–1242.

Unnerstall, J.R., Kuhar, M.J., Niehoff, D.L., Palacios, J.M., 1981. Benzodiazepine receptors are coupled to a subpopulation of gamma-aminobutyric acid (GABA) receptors: evidence from a quantitative autoradiographic study. J. Pharmacol. Exp. Ther. 218, 797–804.

Wisden, W., Laurie, D.J., Monyer, H., Seeburg, P.H., 1992. The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain: I. Telencephalon, diencephalon, mesencephalon. J. Neurosci. 12, 1040–1062.