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Zolpidem binding sites on the $GABA_A$ receptor in brain from human cirrhotic and non-cirrhotic alcoholics

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Abstract

The displacement of [^{3}H]flunitrazepam by unlabelled flunitrazepam or zolpidem was used to assess the affinity and density of sub-types of $GABA_A$ receptors in the superior frontal and primary motor cortices of ten alcoholic, seven alcoholic-cirrhotic and ten matched control cases. The binding was best fitted by a model with a single site for flunitrazepam, but two sites for zolpidem. Neither the patients' age nor the post-mortem interval were significantly correlated with the affinity or density of any of the binding sites. The affinity of all ligands did not differ either between cortical regions or across case groups. Hence, the density of each binding site was analyzed at constant affinity. The densities of flunitrazepam and high-affinity zolpidem binding sites were invariant across cortical regions and case groups. Low-affinity zolpidem binding sites were significantly more dense in the frontal than in the motor cortex of alcoholic cases irrespective of cirrhosis, whereas this regional difference was not significant in control cases.

Keywords: Brain damage; Pathogenesis; Cerebral cortex; (Human); Receptor isoform; Receptor subtype

1. Introduction

Chronic and excessive ingestion of alcohol in human beings is associated with selective neuronal losses and changes in neurotransmitter systems. The brain damage caused by alcohol is both region- and cell-specific: for example, morphometric analysis has revealed a 22% decrease in the number of neurones in the superior frontal cortex of chronic alcoholic cases, as compared with an unaffected area (such as the primary motor cortex) in the same cases or with the homotropic area in non-alcoholic control cases (Kril and Harper, 1989; Kril et al., 1997). In addition, there was a reduction in the mean neuronal surface area in both cortical areas, indicating that shrinkage of these cells had occurred (Kril and Harper, 1989). These findings suggest that specific neuronal populations may be susceptible to damage in chronic alcoholism.

Pharmacological studies in these same brain regions have shown that the density of $GABA_A$ receptor binding sites is significantly greater in the superior frontal cortex of non-cirrhotic alcoholic cases, as compared with either the same region in control cases or with the motor cortex of the same case. This greater binding reflects a higher density of both agonist (high-affinity GABA) and benzodiazepine ('central-type') sites (Tran et al., 1981; Dodd et al., 1992). GABA enhancement of benzodiazepine binding is also altered in the superior frontal cortex of alcoholics (Dodd et al., 1996). Thus, alcoholism may lead to a functional uncoupling of the $GABA_A$/benzodiazepine receptor complex. Post-synaptic $GABA_A$ receptor site densities are greater in alcoholic cases with Wernicke encephalopathy, although in these cases the 'central-type' benzodiazepine site, labelled with flunitrazepam and diazepam, showed equal or lower numbers of binding sites, cf., controls, respectively (Dodd et al., 1996). The affinity of flunitrazepam and diazepam also varied with the severity of disease. Hence, the density of receptor sites and the population of receptor subtypes appears to vary in the frontal cortex of alcoholic cases with and without Wernicke encephalopathy and cirrhosis of the liver.

The $GABA_A$ receptor is a pentameric complex composed of several distinct polypeptide subunits (Nayeem et al., 1994). These have been divided into five classes on the basis of sequence homology. To date, 15 isoforms, designated α_{1-6}, β_{1-4}, γ_{1-3} and δ, a number of which can be

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expressed as splice variants, have been cloned from mammalian and avian brain (see Lewohl et al., 1996). If there are no, or few, restrictions the combination of isoforms in the formation of the receptor complex, many varieties of functional receptor are possible.

In a recent study of human autopsy tissue, competitive reverse transcriptase-polymerase chain reaction (RT/PCR) assays were used to quantify the expression of the α_1, α_2 and α_3 isoforms of the $GABA_A$ receptor in the superior frontal and primary motor cortices of control, uncomplicated alcoholic and alcoholic-cirrhotic cases. The expression of α_1 isoform mRNA was shown to be increased in alcoholics, while that of α_2 and α_3 remained relatively constant (Lewohl et al., 1997). These alterations in gene expression of the α_1 isoform, coupled with the pharmacological changes in the $GABA_A$ receptor, suggest that there is an alteration in $GABA_A$ receptor subtype in the frontal cortex of human alcoholic cases.

Zolpidem is an imidazopyridine (non-benzodiazepine) hypnotic sedative which binds to type I and type II benzodiazepine receptors with differential affinity (Ruano et al., 1993). Type I and type II benzodiazepine receptors are pharmacologically distinct $GABA_A$ receptor subtypes which have been shown to be differentially expressed across the brain (Ruano et al., 1992). Type I receptors are associated with sensorimotor areas and are thought to mediate the sedative and hypnotic effects of benzodiazepine agonists. Type II receptors are expressed in cortical and subcortical regions and are involved in mediating anxiolytic and anticonvulsant effects (Dennis et al., 1988; Perrault et al., 1990).

The affinity of the receptor for zolpidem has been reported to depend on the nature of the α-isoform component present in the receptor complex: α_1-containing receptors bind zolpidem with high affinity, whereas α_2-, α_3- and α_5-containing receptors bind zolpidem with low affinity (Ruano et al., 1992). The aim of the present study was to determine whether zolpidem binding parameters were consistent with an up-regulation in α_1 mRNA expression in the superior frontal and motor cortices of control, alcoholic and alcoholic-cirrhotic cases matched for age and post-mortem delay.

2. Materials and methods

2.1. Patient tissue

The cases and tissue samples on which this study was based were identical with those used for the assay of α-isoform mRNA expression in our earlier report (Lewohl et al., 1997). Consent for the use of autopsy tissue was obtained from the coroner, or by the informed, written authority of the next of kin for hospital necropsies and ethical clearance from the Royal Brisbane Hospital (Protocol 1992/22). The right cerebral and cerebellar hemispheres were collected, slowly frozen in 0.32 M sucrose and stored at −70°C (Dodd et al., 1986). The other hemisphere was fixed in formalin for pathological examination. For each case, information regarding general health, diet, alcohol intake, medication and the presence of alcohol-related diseases such as cirrhosis of the liver, was available. No case had clinical or pathological evidence of neurological or psychiatric disease (apart from alcoholism) prior to death, and none was on prescribed neuroactive medication. As far as could be ascertained from the reports, no patient was a polysubstance or intravenous drug abuser. Cases were divided into groups depending on alcohol intake and the presence of complicating disease. Full liver pathology, which differentiated cirrhotic and non-cirrhotic subjects, was available in all cases. Summary details are shown in Table 1. Controls were teetotal or had low alcohol intake (< 20 g of ethanol per day), whereas alcoholics were defined by a consumption of over 80 g of ethanol per day. All the alcoholics had been abusing the drug for most of their adult lives. More detailed information on liver pathology and cause of death in the individual cases may be found in our earlier report (Lewohl et al., 1997).

2.2. Preparation of crude synaptic membranes

Membranes were prepared as described in Maddison et al. (1987). Tissue pieces were thawed rapidly in 0.32 M sucrose at 37°C (Dodd et al., 1986) and immediately cooled to 4°C. The tissue was homogenized in 10 volumes of ice-cold 0.32 M sucrose using a motor-driven Teflon-glass homogenizer. The homogenate was centrifuged at $750 \times g$ for 10 min at 4°C, the supernatant collected and re-centrifuged at $18\,900 \times g$ for 20 min at 4°C. The pellet was resuspended in 10 volumes of ice-cold distilled water and stored in aliquots at −80°C. On the day of assay, the membranes were thawed and washed four times in ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 4°C). The pellet was resuspended in buffer at a final concentration of 0.2–0.4 mg protein/ml and kept on ice until use.

2.3. Receptor binding assays

Flunitrazepam and zolpidem binding assays were performed together in duplicate, using the same membrane preparation, at 4°C in 50 mM Tris-HCl (pH 7.4) in a total volume of 250 μl. Each tube contained a 50 μl aliquot of the membrane suspension together with 1 nM [^{3}H]flunitrazepam (82.0 Ci/mmol, NEN-Dupont) and varying concentrations of unlabelled flunitrazepam (0.2–200 nM) or zolpidem (0.1 nM–10 μM), respectively. Non-specific binding was measured by the inclusion of 10 μM unlabelled flunitrazepam or zolpidem. The incubation was terminated after 30 min by filtration onto Whatman GF/B glass-fibre filters and immediately washed with 3×1 ml of ice-cold 50 mM Tris-HCl buffer with the aid

Table 1
Case summaries

Group	Age (years)	Sex	Post-mortem delay (h)
1. Controls, $n = 10$	68.0 (54–90)	6 males, 4 females	18.0 (3–56)
2. Uncomplicated alcoholics, $n = 10$	65.5 (36–89)	8 males, 2 females	23.5 (4.75–37)
3. Cirrhotic alcoholics, $n = 7$	58.0 (39–66)	7 males	26.75 (< 6–58)
Combined alcoholics, $n = 17$	63.0 (36–89)	15 males, 2 females	26.75 (4.75–58)

Values are medians, with the range in parentheses. Full details of the cases, including individual age and post-mortem delay values, have been reported elsewhere (Lewohl et al., 1997).

of a Brandel cell harvester (model M-24). Filter-bound radioactivity was measured by liquid scintillation spectrometry using a Beckman LS 3801 β-counter with on-board quench correction. Average efficiency was approx. 33%.

2.4. Protein concentration

Protein concentrations of the membrane suspensions were determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

2.5. Data analysis

Binding data was analyzed by the Scatchard method using the computer programs EBDA and LIGAND (Dodd et al., 1992) to yield K_d and maximal number of binding sites (B_{max}) values by iterative curve fitting (Munson and Rodbard, 1980). [^{3}H]Flunitrazepam bound to a single site, whereas analysis of the zolpidem displacement of [^{3}H]flunitrazepam binding showed that a two-site model gave a statistically significant improvement of fit over a one-site model, $P < 0.01$ in all cases. However, attempts to fit a three-site model were always unsuccessful.

Data analysis was carried out in stages. First, the individual K_d values for flunitrazepam binding were derived. Analysis of variance on the logarithms of these values (Bürgisser, 1984) showed there were no significant differences between these values either when different cortical regions in the same cases or the homotropic regions in different case groups were compared (Table 2). The geometric mean flunitrazepam K_d was then calculated (Dodd et al., 1992). The displacement curves from the flunitrazepam and zolpidem assays were analyzed together, using the geometric mean flunitrazepam K_d as a fixed parameter. The binding model used was

K_d^H flunitrazepam = K_d^L flunitrazepam, but

K_d^H zolpidem ≠ K_d^L zolpidem.

where K_d^H and K_d^L represent the dissociation constants for the high- and low-affinity sites, respectively.

Analyses of variance on the logarithms of either zolpidem K_d value showed that there were no significant differences when either different cortical regions from the same cases, or homotropic regions from different case groups, were compared (Table 2). Although cross-checks showed that the statistical analysis of the B_{max} values was unaffected by the final stage of analysis (below), the best estimates of absolute zolpidem B_{max} values are obtained when these are calculated at constant K_d, since K_d and B_{max} values are mathematically correlated (Bürgisser, 1984). Obtaining the best possible estimates of zolpidem B_{max} values was an important aspect of this study (vide infra). Hence, the overall geometric mean zolpidem K_d values were computed, and each pair (zolpidem plus flunitrazepam) of [^{3}H]flunitrazepam displacement curves re-analyzed at fixed K_d values for the two ligands.

It is a consequence of this analytical approach that for each binding assay,

B_{max}^H zolpidem + B_{max}^L zolpidem = B_{max} flunitrazepam,

where B_{max}^H zolpidem and B_{max}^L zolpidem represent the high- and low-affinity B_{max} values for zolpidem, respectively.

The overall geometric mean values used in these analyses were:

K_d^H flunitrazepam = K_d^L flunitrazepam = 1.33 nM;

K_d^H zolpidem = 14.7 nM;

K_d^L zolpidem = 4.39 μM.

Table 2
Apparent dissociation constants for flunitrazepam and zolpidem

Case	K_d (nM)		K_d (μM)
	Flunitrazepam	Zolpidem high-affinity site	Zolpidem low-affinity site
Controls			
Frontal	1.31 ± 0.16	19.1 ± 2.4	6.59 ± 2.85
Motor	1.35 ± 0.15	11.9 ± 2.4	6.38 ± 0.16
Uncomplicated alcoholics			
Frontal	1.32 ± 0.16	17.0 ± 0.9	3.88 ± 1.83
Motor	1.37 ± 0.19	16.3 ± 2.3	3.44 ± 1.79
Cirrhotic alcoholics			
Frontal	1.45 ± 0.13	12.2 ± 5.2	5.05 ± 3.43
Motor	1.17 ± 0.19	11.6 ± 1.5	2.10 ± 1.18

3. Results

As Table 1 shows, the case groups used in this study were reasonably well matched for age at death, and neither the age nor the post-mortem delay values varied significantly on either parametric or non-parametric tests (Lewohl et al., 1997). Sample sizes were too small to allow the effects of these variables to be assessed in the separate case groups. However, analysis of the regression of binding-site B_{max} values on either age or post-mortem delay in both brain areas from the combined cases showed that no correlation with either variable was statistically significant (Fig. 1). When these calculations (see Fig. 1 legend) were repeated using non-parametric statistics (Spearman's rank correlation coefficient, r_S; Kendall's τ), it was again found that there was no significant effect of age or post-mortem delay on the binding values, $P > 0.1$ in all cases. The matching of the case groups and the lack of these correlations meant that neither age nor post-mortem delay should have confounded subsequent analyses.

When flunitrazepam B_{max} data were analyzed at a constant K_d (Fig. 2), there was no significant variation either between cortical areas in the same case groups ($F(1,24) = 0.30$; $P = 0.59$), or between equivalent areas across case groups ($F(2,24) = 0.54$; $P = 0.59$).

In general, the pattern of high-affinity zolpidem binding-site densities was similar to that of flunitrazepam (Fig. 3, cf., Fig. 2), whereas low-affinity zolpidem B_{max} showed a somewhat different pattern (Fig. 4). Moreover, for each of these parameters the two alcoholic groups were strik-

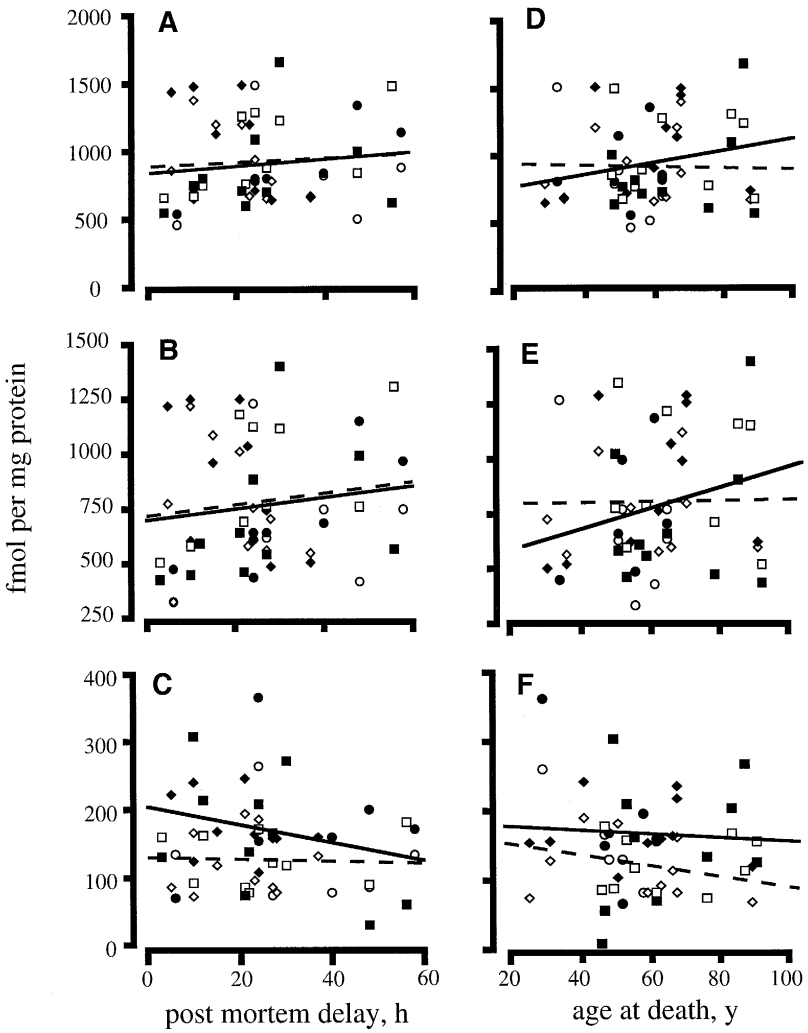

Fig. 1. Effect of post-mortem delay and age at death on the density of flunitrazepam, zolpidem high-affinity and zolpidem low-affinity binding sites. Regression analysis was performed for each ligand within areas across the combined cases. Since none of the correlations was statistically significant (vide infra), the least-squares lines are shown for indicative purposes only. A–C, regression on post-mortem delay; D–F, regression on age; solid lines, average regression in superior frontal cortex (solid symbols); broken lines, average regression in primary motor cortex (open symbols); ■ □, controls, $n = 10$; ♦ ◇, uncomplicated alcoholics, $n = 10$; ● ○, cirrhotic alcoholics, $n = 7$; AD, flunitrazepam B_{max}; BE, high-affinity zolpidem B_{max}; CF, low-affinity zolpidem B_{max}. Correlation coefficients: A frontal, $r(25) = 0.063$, $P = 0.608$; A motor, $r(25) = 0.120$, $P = 0.533$; B frontal, $r(25) = 0.133$, $P = 0.367$; B motor, $r(25) = 0.139$, $P = 0.435$; C frontal, $r(25) = 0.249$, $P = 0.101$; C motor, $r(25) = 0.042$, $P = 0.843$; D frontal, $r(25) = 0.217$, $P = 0.356$; D motor, $r(25) = 0.031$, $P = 0.913$; E frontal, $r(25) = 0.252$, $P = 0.218$; E motor, $r(25) = 0.017$, $P = 0.938$; F frontal, $r(25) = 0.059$, $P = 0.690$; F motor, $r(25) = 0.281$, $P = 0.215$.

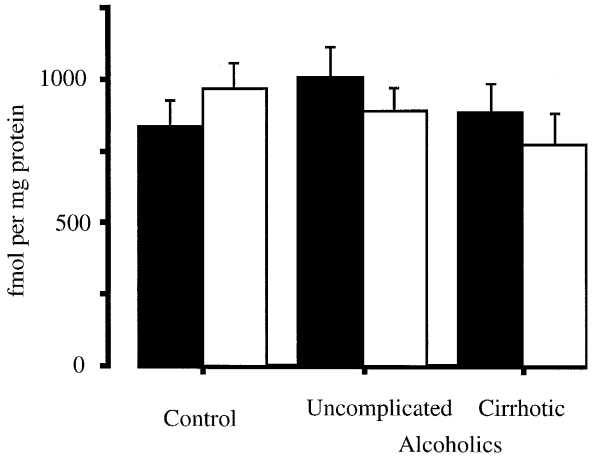

Fig. 2. Density of flunitrazepam binding sites in different cortical areas. The density of binding sites was calculated as described in Section 2; shaded columns, superior frontal cortex; open columns, primary motor cortex. Statistical analyses are given in the text. Values are means ± S.E.M. of the numbers of cases shown in Table 1 for each group.

ingly similar, and no statistical test distinguished between them. All analyses of variance were therefore re-computed for two case groups, i.e., controls vs. combined alcoholic cases. In the overall analysis of zolpidem B_{max}, the Binding Site × Cortical Region Interaction was significant ($F(1,25) = 7.900$; $P = 0.0095$). This difference in pattern came about because for the high-affinity site, the Cortical Region × Group term approached significance ($F(1,25) = 3.6253$; $P = 0.069$), whereas for the low-affinity site, the equivalent term was not significant but the Main Effect for Cortical Region was ($F(1,25) = 5.746$; $P = 0.024$). Inspection showed that for the high-affinity site, frontal B_{max} was greater than motor B_{max} for all alcoholics, while the reverse was true in controls (Fig. 3). In contrast, low-affinity zolpidem B_{max} was always greater in frontal than in motor cortex (Fig. 4); moreover, by post-hoc Newman-Keuls test this regional difference was not statistically significant in controls, but was significant in the combined

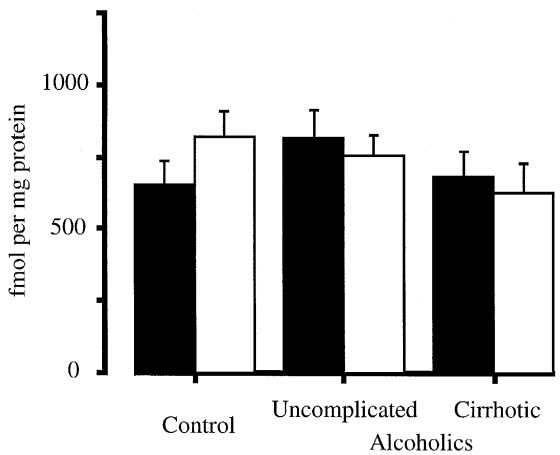

Fig. 3. Density of high-affinity zolpidem binding sites in different cortical areas. Legend as for Fig. 2.

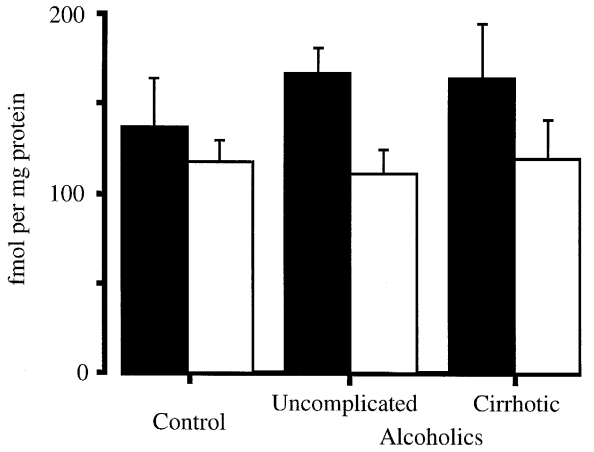

Fig. 4. Density of low-affinity zolpidem binding sites in different cortical areas. Legend as for Fig. 2.

alcoholic cases ($P < 0.05$). When the two alcoholic groups were separated, no within-group comparison was statistically significant. A tendency towards significant regional difference in uncomplicated alcoholics was suggested by a significant Tukey LSD test ($P < 0.05$). Power analysis indicated that if the mean and standard deviation values obtained are true estimators of the population parameters, a case-group size of approximately 13 would be required to achieve statistically significant regional differences which would occur in both groups of alcoholics. That is, at least 12 further cases would be needed to attain this result. Unfortunately, such additional case numbers, especially of the six further cirrhotic alcoholics required, were unavailable.

4. Discussion

The aim of the present study was to determine the affinity and density of $GABA_A$ receptors in the frontal and motor cortices of control, alcoholic and alcoholic-cirrhotic cases, using flunitrazepam and zolpidem as displacers of [^{3}H]flunitrazepam. We found no difference in affinity or density for flunitrazepam or high-affinity zolpidem binding between the frontal and motor cortices within case groups or in the same regions between case groups. However, an area difference in low-affinity zolpidem binding site density was more pronounced in alcoholics and alcoholic-cirrhotic cases than in controls. This appears to reflect an increase in the density of type II benzodiazepine receptors in the frontal cortex of alcoholic cases irrespective of cirrhosis.

The tissue used in this study was collected, stored and prepared under conditions which have previously been shown to be optimal for the preservation of receptor-site integrity (Dodd et al., 1986). Active preparations that have been isolated from human brain tissue up to 24 h post mortem exhibit a variety of activities, including respira-

tion, K^+ ion accumulation and neurotransmitter release and uptake (Dodd et al., 1988). For receptor binding analysis, it is not necessary to isolate metabolically or functionally active synaptosomes and therefore tissue can be collected over a wider range of post-mortem intervals. A number of studies have shown that there is little change in protein content or neurotransmitter receptor binding properties in control or alcoholic cases up to 100 h post mortem (Tran et al., 1981; Freund and Ballinger, 1988, 1989; Kril et al., 1988). Other factors such as age and agonal state of the patient may influence receptor binding properties. However, no correlation was found between age and [^{3}H]flunitrazepam binding in cases ranging from 30 to 90 years of age (Freund and Ballinger, 1988, 1989).

Although receptor binding properties are not generally influenced by either age or post-mortem delay, it cannot be assumed that this is true for all receptor species. The cases for this study were carefully selected on the basis of agonal state and matched for age and post-mortem delay. Accordingly, there was no correlation between the density of flunitrazepam and zolpidem binding sites and either age or post-mortem delay.

Flunitrazepam has been reported to bind to $GABA_A$ receptors containing α_1, α_2, α_3 and α_5 subunits with equal affinity, whereas zolpidem has been found to be highly selective for receptors containing the α_1 isoform in recombinant studies. The lack of a change in flunitrazepam affinity or density reported here is consistent with previously reported findings (Dodd et al., 1992) and is in agreement with the idea that flunitrazepam binds to all benzodiazepine receptors with equal affinity.

Zolpidem binding was best fitted by a two-site model in all cases. High-affinity and low-affinity zolpidem receptors have been thought to correspond to type I and type II benzodiazepine receptors, respectively. In some areas of the rat brain, three zolpidem binding sites have been identified. These have been designated high-, low- and very-low-affinity, and have K_d values of 10–20 nM, 200–300 nM and 4–10 μM, respectively (Ruano et al., 1992). The average zolpidem K_d value across all areas and case groups in human cerebral cortex was found to be 14.7 ± 1.1 nM for the high-affinity site, while the low-affinity site gave a K_d value of 4.4 ± 1.1 μM. Although the average K_d for the human cortical high-affinity site corresponds to the equivalent parameter for the rat, the average affinity for the human cortical low-affinity binding site in this study is in the range of the very-low-affinity binding site previously detected only in the rat hippocampus. The reason for this discrepancy is unclear. No sample could be analyzed successfully for three binding sites nor was there any evidence for the intermediate range, low-affinity binding site in these samples.

We have recently reported that α_1 isoform mRNA expression was markedly increased in the frontal and motor cortices of alcoholic and alcoholic-cirrhotic cases with respect to controls, on the basis of a precise RT/PCR quantitation method (Lewohl et al., 1997). The cases used in the present receptor binding study were identical to those used for mRNA quantitation. In consequence, if the affinity of human high-affinity zolpidem binding sites were dependent solely on their α_1 content, an increase in the density in high-affinity zolpidem binding sites would have been expected here.

In the rat brain, the topographical pattern of $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor mRNA has been shown to correspond to high-affinity zolpidem binding as well as to ethanol-sensitive GABA responses and is thought to reflect the density of the type I benzodiazepine receptor subtype (Duncan et al., 1995; Criswell et al., 1993, 1995). If this argument can be applied to human brain, the reported increase in α_1 mRNA should have been accompanied by an increase in the density of zolpidem-sensitive receptors. Rather, the results presented here showed an increase in low-affinity zolpidem binding sites which correspond to type II benzodiazepine receptors. In recombinant studies these receptors can contain α_2, α_3 or α_5 subunits (Pritchett et al., 1989; Pritchett and Seeburg, 1990). However, the affinity of the low-affinity zolpidem binding site in human cortex differed markedly from that of the rat type II benzodiazepine site and it is thus impossible to predict the isoform composition of these receptors.

GABA enhancement of benzodiazepine binding in human alcoholic cases is increased in the frontal cortex with respect to the motor cortex of the same case and the homotropic region in control cases (Kril et al., 1988). Recombinant receptor analyses have shown that receptors containing the α_3 isoform in combination with $\beta_1\gamma_2$ show a greater GABA enhancement of benzodiazepine binding (Pritchett et al., 1989). The results presented here are consistent with this finding and may reflect an increase in α_3-containing type II benzodiazepine receptors.

Devaud et al. (1995) found that in rats treated with ethanol for 14 days there were small but reproducible increases in high-affinity [^{3}H]zolpidem binding site density in the cerebral cortex and cerebellum, with no change in affinity. However, in animals treated identically, mRNA expression of the α_1 isoform of the $GABA_A$ receptor was decreased with respect to controls. Hence there was no apparent correlation between α_1 mRNA expression and high-affinity zolpidem binding (Devaud et al., 1995).

To our knowledge, there has been no published study in which the measured changes in α_1 gene expression have been correlated with relevant changes in high-affinity zolpidem binding. This implies that zolpidem binding properties are modulated by other classes of $GABA_A$ receptor isoforms as well as the α subunit class. There is some evidence to suggest that the relative proportion of the γ_2 short (γ_{2S}) and γ_2 long (γ_{2L}) splice variants influences high-affinity zolpidem binding. In the rat brain, areas which had high densities of [^{3}H]zolpidem binding sites also had a greater amount of the γ_{2L} splice variant compared with the γ_{2S} splice variant. Also, rat brain regions

that strongly express $\alpha_1\beta_2\gamma_2$s mRNA have low levels of high-affinity [^{3}H]zolpidem binding sites (Duncan et al., 1995). In a recent study, a point mutation in the human γ_2 splice variant was associated with a profound alteration in the pharmacological properties of the receptor. For example, GABA enhancement of the binding of diazepam and other benzodiazepines was doubled, whereas the response to zolpidem and other ligands selective for type I benzodiazepine receptors was halved. Hence, the γ_2 isoform appears to play a specific role in determining the efficacy of benzodiazepines agonists (Mihic et al., 1994). The γ_{2L} splice variant also influences the action of ethanol at the $GABA_A$ receptor complex, although no difference has been reported in the relative proportions of γ_{2S} and γ_{2L} between areas sensitive and insensitive to ethanol (Sigel et al., 1993; Criswell et al., 1993). These confounding effects of α_1 and γ_2 subunits on high-affinity zolpidem binding may make it an unsuitable ligand with which to analyze $GABA_A$ receptor function in alcoholism, where there is the potential for both α_1 and γ_2 isoform expression to be altered.

In conclusion, we report area differences in zolpidem low-affinity binding site density in alcoholic cases as compared with controls, with no change in affinity. However, there were no changes in flunitrazepam or zolpidem high-affinity site density or affinity in the frontal and motor cortices within case groups or in the equivalent regions between case groups.

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