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Decreases in peripheral-type benzodiazepine receptors in postmortem brains of chronic schizophrenics

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Summary. We measured the peripheral-type benzodiazepine receptors (PBRs), a marker of gliosis, in 26 brain areas (cerebral cortex, thalamus and extrapyramidal system) of the postmortem brains of 13 chronic schizophrenics and 10 controls, using [^{3}H] PK 11195 as a ligand for the receptor assay. The specific [^{3}H] PK 11195 binding was significantly decreased in three brain areas (superior parietal cortex, primary visual area and putamen) of schizophrenics, although there were no changes in the binding in the other brain areas. Scatchard analysis revealed that there were decreases in both the Bmax and Kd of [^{3}H] PK 11195 binding in the brain areas. These results were almost in accordance with a number of neuropathological studies reporting that there was no change or reduction in glial cells in the brain regions of schizophrenics and suggested that the decreased density of PBRs in the brain may be involved in the pathophysiology of schizophrenia, associated with reduced production of neurosteroids coupled to PBRs.

Keywords: Peripheral-type benzodiazepine receptors, schizophrenia, postmortem human brain, [^{3}H] PK 11195, radioreceptor binding.

Introduction

The peripheral-type benzodiazepine receptors (PBRs) differ from the central-type benzodiazepine receptors coupled with The $GABA_A$/chloride ionophore complex in anatomical distribution, subcellular location and physiological functions (Parola et al., 1993). The PBRs show an ubiquitous distribution in the central nervous system (CNS) as well as in the peripheral tissues (De Souza et al., 1985; Kurumaji and Toru, 1996a). It has been well established that the receptors localize in the mitochondrial outer membranes of the adrenal gland (Anholt et al., 1986; Antkiewicz-Michalik et al., 1988) and

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that they mediate cholesterol transport from the outer to the inner mitochondrial membrane and regulate the biosynthesis of steroids (Krueger and Papadopoulos, 1990). Several endogenous compounds, such as diazepam binding inhibitor (DBI) and its processing products (Papadopoulos et al., 1990), play a role as agonist to PBRs. The cDNAs of PBRs proteins have been cloned for the rat (Sprengel et al., 1989), bovine (Parola et al., 1991) and human (Rind et al., 1991) and the deduced amino acid sequences show that the 18kDa protein is hydrophobic with five potential transmembrane regions (Sprengel et al., 1989). Although PBRs can be labelled with benzodiazepine derivatives, such as Ro5-4864, as well as with isoquinoline carboxamides, such as PK 11195 (Awad and Gavish, 1987), PK 11195 and Ro5-4864 may bind to overlapping but not identical sites on the PBRs (Farges et al., 1993). PK 11195 has high affinity for the receptor in all species (Awad and Gavish, 1987; Parola et al., 1993) unlike the species variation in ligand affinity observed for Ro5-4864.

The PBRs are mainly localized on astroglial cells in the rat brain (Itzhak et al., 1995) and are also involved in steroid genesis in the brain mitochondrial preparation (Basil and Skolnick, 1986; McCauley et al., 1995) and C6-2B glioma cells (Papadopoulos et al., 1992). Neurosteroids putatively elicit potent short-term allosteric modulatory effects on the action of GABA at $GABA_A$ receptors (Majewska, 1992), which may produce an anxiolytic effect (Auta et al., 1993) and long-term genomic-mediated effects acting at intracellular steroid receptors (McEwen, 1991). The density of the receptors increase in regions of the brain which have undergone a primary lesion, being associated with glial proliferations (Benavides et al., 1987; Stephenson et al., 1995). In human brains, increases in PBRs have been demonstrated in lesioned areas in a number of neuropathological states, such as Huntington's disease (Schoemaker et al., 1981) and Alzheimer's disease (Owen et al., 1983; McGeer et al., 1988). In schizophrenic brains, neuropathological studies demonstrated no change in the glial cell counts in the cerebral cortices (Benes et al., 1986; Benes and Bird, 1987; Crow et al., 1989; Roberts et al., 1987), the limbic system (Falkai and Bogerts, 1986) and the basal ganglia (Pakkenberg, 1990), and reduced glial densities in the mediodorsal thalamic nucleus and the nucleus accumbens (Pakkenberg, 1990). However, no study has examined PBRs in schizophrenic brains.

In the present study, we examined [^{3}H] PK 11195 binding, labelling PBRs, in 26 brain areas of postmortem brains of chronic schizophrenics to clarify whether or not PBRs are involved in the pathophysiology of schizophrenia. We observed a significant decrease in the specific [^{3}H] PK 11195 binding in three areas of the postmortem brains and then performed Scatchard analysis of the brain areas.

Materials and methods

Subjects and brain dissection

The postmortem brain specimens used were from 13 schizophrenic patients (eight males and five females) and 10 control subjects free from neurological disease (seven males and three females) whose details were described in a previous report (Toru et al., 1988). None

of the subjects had been ventilated before death. All the schizophrenic patients, based on chart review, met the diagnosis criteria for schizophrenia as contained in DSM-IV (Diagnostic and statistical manual of mental disorders, fourth edition). Seven of 13 schizophrenics were "off-drug" cases, who had not received antipsychotics from more than 40 days before death. Four of 13 schizophrenics had taken benzodizepines, chlordiazepoxide or nitrazepam, until immediately before death. The age range of the schizophrenic patients was 41–75 years (mean ± SEM, 60.1 ± 2.7 years) and that of the controls was 52–74 years (66.7 ± 2.7 years); the difference was not statistically significant by the Mann-Whitney U test. The interval from death to freezing of the schizophrenic brains ranged from 3.2 to 24.0 hours (mean ± SEM, 11.3 ± 2.1 hours) and that of the controls from 1.7 to 13.0 hours (5.3 ± 1.2 hours); the former wwas significantly greater than the latter by the same statistical method ($p < 0.05$). The storage time at −80°C was not different between the schizophrenics and controls.

Tissue preparation and receptor binding assay

The frozen brains were sectioned as previously reported (Toru et al., 1988). Twenty-four hours before dissection, brains stored in airtight packages at −80°C were transferred to a cold box maintained at −15°C to allow sectioning. The frozen brains were sectioned coronally in 10-mm thick slices, mounted on a freezing microtome, and the frozen blocks were cut into 1.5-mm thick slices. Each specific brain area was dissected out from these slices in a cold box at −15°C. Dissected tissues were homogenized with a glass-Teflon homogenizer in three volumes of chilled 0.32 mol/L sucrose and stored at −80°C prior to being assayed.

The [^{3}H] PK 11195 binding assay and membrane preparation were performed using a previously reported method (Kurumaji and Toru, 1996a). The brain homogenates were thawed and homogenized in 50 volumes of Dulbecco's phosphate buffered saline (PBS) (Nissui Pharmaceutical Company, Tokyo, Japan) (pH 7.4) at 4°C with the use of an Ultra-Turrax polytron for 15 seconds at half maximal setting (13,500 rpm). The homogenate was centrifuged at 40,000 × g for 30 minutes, and the pellet was resuspended in 50 volumes of PBS. After the same centrifugation, the pellet was resuspended in 20 volumes of PBS. The tissue membrane and 1nM [^{3}H]PK 11195 (85.5 Ci/m mol, New England Nuclear) in 0.6 ml of PBS were incubated for 90 minutes at 4°C. The incubations were terminated by filtration through Whatman GF/B filters presoaked with 0.1% polyethyleneimine, and followed by three washes with 4 ml of chilled PBS. Nonspecific binding was defined by 10 μM unlabelled PK 11195. For the kinetic analysis of the binding parameters, a regularly increasing concentration of [^{3}H]PK 11195, from 0.2 to 20 nM, was used. Kinetic parameters such as Bmax and Kd were calculated from the first-order equation analysis.

Protein concentrations were determined according to the method of Lowry et al. (1951).

Data analysis

Data were expressed as mean ± SEM values. Data from the schizophrenics and controls were compared statistically by means of multiple regression analysis, the regressor variables being diagnosis, age at death, and interval from death to freezing. This analysis is equivalent to the group comparison of schizophrenics and controls adjusted for age at death and interval from death to freezing (Armitage and Berry, 1987).

Results

A statistically significant decrease in the specific [^{3}H]PK11195 binding was observed in two cerebral cortices, the superior parietal cortex and the primary visual area, of the schizophrenic brains, compared to those of the controls (Table 1). In the extrapyramidal system and thalamus, there was

Table 1. Specific binding of [^{3}H]PK 11195 binding in the cerebral cortex

	Controls	Schizophrenics
Prefrontal cortex		
Medial frontal cortex	96.5 ± 6.2 (7)	100.9 ± 3.1 (11)
Orbitofrontal cortex	104.4 ± 6.7 (7)	107.3 ± 5.2 (12)
Eye-movement area	70.8 ± 6.4 (9)	73.0 ± 7.7 (12)
Orbital cortex	100.3 ± 6.8 (8)	88.1 ± 5.5 (9)
Precentral area		
Premotor area	88.8 ± 4.3 (9)	76.1 ± 4.0 (9)
Temporal cortex		
Superior temporal cortex	86.1 ± 6.1 (10)	88.0 ± 4.0 (9)
Medial and inferior temporal cortex	72.1 ± 4.5 (10)	69.9 ± 3.6 (9)
Lateral occipitotemporal cortex	86.5 ± 7.2 (10)	69.3 ± 2.3 (7)
Parietal cortex		
Somesthetic cortex	83.5 ± 10.5 (8)	67.1 ± 4.1 (11)
Superior parietal cortex	99.4 ± 8.4 (10)	58.1 ± 6.8 (10)**
Supramarginal cortex	69.3 ± 4.7 (10)	69.4 ± 2.8 (9)
Angular cortex	66.5 ± 9.2 (10)	64.2 ± 3.6 (11)
Occipital cortex		
Visual area 1	56.5 ± 5.5 (7)	43.4 ± 1.6 (11)**
Visual area 2 and 3	80.0 ± 4.6 (9)	77.8 ± 3.7 (7)

Values in fmoles/mg protein are shown as mean ± SEM (n). Binding assays were carried out in a final concentartion of 1 nM of [^{3}H] PK 11195. Each comparison was performed by means of multiple regression analysis adjusted for age at death and interval from death to freezing. ** $p < 0.01$ vs. controls

Table 2. Specific binding of [^{3}H]PK 11195 binding in the extrapyramidal system and thalamus

	Controls	Schizophrenics
Extrapyramidal system		
Caudate	79.5 ± 7.9 (10)	70.3 ± 5.4 (11)
Putamen	65.0 ± 6.5 (9)	41.6 ± 2.5 (12)*
Pallidum externa	79.5 ± 7.6 (8)	70.6 ± 4.5 (9)
Pallidum interna	82.0 ± 10.7 (8)	75.5 ± 6.2 (8)
Substantia nigra	88.0 ± 6.8 (9)	107.3 ± 9.0 (10)
N. ruber	82.2 ± 7.6 (9)	106.0 ± 4.9 (9)
N. subthalamicus	72.2 ± 9.5 (4)	121.2 ± 17.8 (6)
Thalamus		
N. anterior thalami	76.5 ± 6.1 (6)	62.1 ± 4.3 (7)
N. lateralis anterior	83.7 ± 11.1 (9)	71.3 ± 12.8 (7)
N. lateralis posterior	102.9 ± 10.9 (10)	87.7 ± 5.4 (10)
N. dorsomedialis	141.8 ± 10.9 (9)	132.7 ± 10.5 (9)
N. centralis medialis	129.7 ± 7.2 (9)	144.1 ± 6.5 (9)

Values in fmoles/mg protein are shown as mean ± SEM (n). Binding assays were carried out in a final concentartion of 1 nM of [^{3}H] PK 11195. Each comparison was performed by means of multiple regression analysis adjusted for age at death and interval from death to freezing. * $p < 0.05$ vs. controls

a statistically significant reduction in the binding to putamen of the schizophrenics (Table 2).

Statistically significant decreases in both Bmax and Kd were found in the superior parietal cortex of the schizophrenics. The reduction in Bmax was also statistically significant in the off-drug cases, and the decrease in Kd was statistically significant in the on-drug cases (Table 3 and Fig. 1). There were decreases in Bmax as well as Kd in the primary visual area and putamen. A statistically significant reduction in Kd was observed in the visual area of total schizophrenics and of off-drug cases (Table 3).

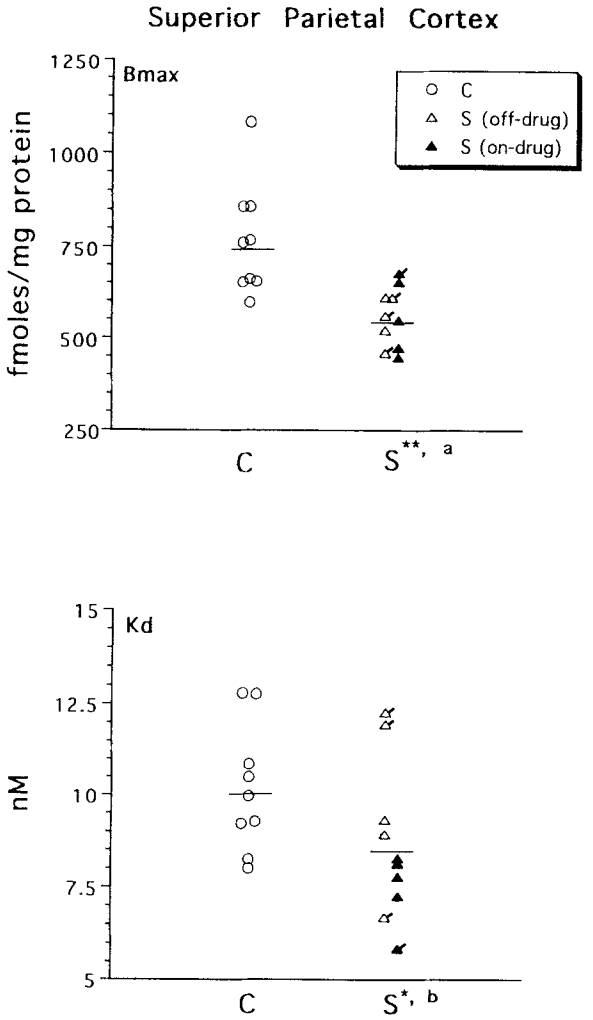

Fig. 1. Bmax and Kd of [^{3}H] PK 11195 binding in the superior parietal cortex of controls (C) and schizophrenics (S). (△) "Off-drug" cases, who had received no antipsychotics for more than 40 days before death; (▲) "On-drug" cases, who had been treated with antipsychotics until immediately before death. (△ or ▲) Cases treated with benzodiazepines until immediately before death. *Horizontal bars* indicate mean values. Each comparison was carried out by means of multiple regression analysis adjusted for age at death and interval from death to freezing. *$p < 0.05$ vs. controls, **$p < 0.01$ vs. controls. [a]$p < 0.05$ Off-drug cases vs. controls, [b]$p < 0.01$ On-drug cases vs. controls

Table 3. Scatchard analysis of [^{3}H] PK 11195 binding

	Controls	Schizophrenics		
		Total	Off-drug	On-drug
Superior parietal cortex				
Bmax	767.2 ± 50.2 (9)	556.3 ± 25.8 (10)**	552.4 ± 28.6 (5)*	560.2 ± 46.4 (5)
Kd	10.2 ± 0.58 (9)	8.7 ± 0.66 (10)*	9.2 ± 1.1 (5)	7.9 ± 0.23 (5)**
Visual area 1				
Bmax	862.8 ± 64.0 (7)	761.3 ± 61.6 (7)	824.4 ± 59.7 (5)	603.8 (2)
Kd	14.9 ± 1.5 (7)	10.4 ± 1.2 (7)*	9.8 ± 1.6 (5)*	11.7 (2)
Putamen				
Bmax	447.7 ± 43.6 (4)	368.0 ± 15.4 (4)	369.2 ± 21.7 (3)	364.3 (1)
Kd	5.9 ± 0.5 (4)	4.0 ± 0.7 (4)	4.2 ± 0.7 (3)	3.4 (1)

Bmax (maximum number of binding, fmoles/mg protein) and Kd (affinity of binding, nM) are shown as mean ± SEM (n). Each comparison was carried out by means of multiple regression analysis adjusted for age at death and interval from death to freezing. *$p < 0.05$ vs. controls, **$p < 0.01$ vs. controls

In the superior parietal cortex of schizophrenics, there were no differences between benzodiazepine-free schizophrenics (BZ-off; n = 6) and benzodiazepine-intake patients (BZ-on: n = 4) either in Bmax (mean ± SEM; fmoles/mg protein) (BZ-off: 542 ± 32, BZ-on: 577 ± 46) or in Kd (mean ± SEM; nM)(BZ-off: 8.29 ± 0.3, BZ-on: 9.21 ± 1.7)(Fig. 1).

Discussion

The present study demonstrated prominent decreases in the specific [^{3}H]PK 11195 binding in three brain areas (superior parietal cortex, visual area I and putamen) of the postmortem brains of chronic schizophrenics. Scatchard analysis revealed decreases in Bmax as well as Kd of [^{3}H] PK 11195 binding in the brain areas of the schizophrenics. The reduction in Bmax in the superior parietal cortex and the decrease in Kd in the primary visual area were also marked in the off-drug cases of the schizophrenics, while the decrease in Kd in the superior parietal cortex was statistically significant in the on-drug cases. In rat brains, chronic treatment with antipsychotic drugs, such as haloperidol and chlorpromazine, produced an increase in Bmax of [^{3}H] PK 11195 binding, but not Kd, in the olfactory bulb (Kurumaji et al., 1996b) and in the cerebral cortex (Gavish et al., 1988; Gavish and Weizman, 1989). Thus, it is unlikely that medication with antipsychotic drugs produced the decreases in Bmax and Kd of [^{3}H] PK 11195 binding in the schizophrenic brains. Moreover, the four schizophrenic patients had taken benzodiazepines (chlordiazepoxide and nitrazepam), which have a weak affinity for PBRs (Hertz, 1993), until immediately before death. Although the benzodiazepines remaining in the binding tissue membranes possibly resulted in an increase in Kd of the binding and prolonged intake of the drugs may have contributed to alterations in the binding density, there was no consistent change in the parameter of the four patients (see Fig. 1).

The PBRs are mainly localized on astroglial cells in the rat brain (Itzhak et al., 1995). Astrocytes exhibit changes in response to almost every type of injury or disease in the CNS. Immediately after insults, the glial cells undergo both hyperplasia and hypertrophy in an attempt to repair the damage. As the tissue repair response matures, astocytic bodies diminish in number, whereas fibrillary gliosis become more prominent. An increase in the density of PBRs was observed in a primary lesion of neurodegenerative diseases, such as Alzheimer's disease (Owen et al., 1983; McGeer et al., 1988) and Huntington's disease (Schoemaker et al., 1981). Although an increase in the chronic fibrillary gliosis remains controversial in schizophrenic brains (Bruton et al., 1990; Fisman, 1975; Stevens, 1982), glial cell densities were unchanged in the cerebral cortices (Benes et al., 1986; Benes and Bird, 1987; Crow et al., 1989; Roberts et al., 1987), the hippocampus (Falkai and Bogerts, 1986) and the ventral pallidum (Pakkenberg, 1990) and were reduced in the mediodorsal thalamic nucleus and the nucleus accumbens (Pakkenberg, 1990). In the present study, decreases in three brain areas and minimal changes in the other areas were found in the number of [^{3}H] PK 11195 binding of the postmortem brains of schizophrenic patients. Considering that the capacity of astrocytes to

react with proliferation and hypertrophy develops during the last trimester of gestation, these results also indicate that there was no progressive neuropathological process in the schizophrenics after birth. The density of PBRs in rat brains increased markedly from 0 to 14 days of age during postnatal development (Kurumaji and Toru, 1996a). It is possible that the increase in the density of PBRs during development is affected in the schizophrenic brains.

Because it has been reported that PBRs play an important role in steroid genesis in the CNS (McCauley et al., 1995; Papadopoulos et al., 1992), the reduced density of PBRs might be parallel with a decreased production of neurosteroids, which produces impairments of $GABA_A$ receptor functions (Majewska, 1992) and genomic-effects at the steroid receptors (McEwen, 1991). In addition, Doble et al. (1987) demonstrated by a method of quantitative autoradiographic receptor binding that [^{3}H] PK 11195 bindings in the human brain were distributed heterogeneously and uniquely in the gray matter, suggesting that these sites may play an as yet unknown role in human brain function.

However, the mechanism whereby the decreases in Kd of [^{3}H] PK 11195 binding was induced in the schizophrenic brains is also unclear. It is less likely that endogenous agonists remaining in the binding homogenates after two washings with PBS modulated the binding parameter. The changes in the parameter in the schizophrenics, by any chance, could be attributed to unknown alterations in the conformation and/or structure of PBRs.

In conclusion, there were decreases in the Bmax and Kd of [^{3}H] PK 11195 binding in the superior parietal cortex, primary visual area and putamen of the postmortem brains of chronic schizophrenics. It appears that these results are in agreement with the previous neuropathological studies of schizophrenic brains. The reduced density of PBRs may be involved in the pathophysiology of schizophrenia, associated with dysfunctions of neurosteroids. Further studies are required to clarify the regulation mechanism of PBRs in human brains.

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