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Strain differences in the distribution of N-Methyl-D-Aspartate and Gamma (γ)-aminobutyric acid-A receptors in rat brain

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Abstract

Aims—Previous studies have shown that the Wistar-Kyoto (WKY) rat strain exhibits depressive symptoms such as anhedonia, psychomotor retardation, ambivalence and negative memory bias following exposure to stress. Given the involvement of excitatory glutamate and inhibitory gamma (γ)-aminobutyric acid (GABA) signaling pathways in influencing depressive behavior, the present study investigated strain differences in the distribution of central N-methyl-D-aspartate (NMDA) and GABA_A receptor sites in WKY compared to their inbred counterpart, Wistar (WIS) rats.

Main methods—Quantitative autoradiographic analysis was used to map the binding and distribution of NMDA and GABA_A receptors in various brain regions in WKY and WIS rats.

Key findings—Results indicated a significant difference between the two strains. Lower NMDA receptor binding was found in the anterior cingulate cortex, caudate putmen, nucleus accumbens, CA1 region of the hippocampus and the substantia nigra pars reticulata in WKY compared to WIS rats. Conversely, higher GABA_A receptor binding was found in the amygdala, caudate putmen, dentate gyrus, CA2 and CA3 fields of the hippocampus, periaqueductal gray and substantia nigra pars reticulata in WKY compared to WIS rats.

Significance—Given that these two rat strains differ in their behavioural, endocrine and neurochemical profile, the observed strain differences in NMDA and GABA_A receptor binding suggests that these two neurotransmitter systems may be involved in the depressive and stress sensitive phenotype of the WKY rat strain.

Keywords

N-Methyl-D-Aspartate Receptors; Gamma (γ)-aminobutyric acid Receptors; Wistar Kyoto Rat; Quantitative Autoradiography

Introduction

The Wistar Kyoto (WKY) rat strain has been proposed as an animal model of depressive behavior because it exhibits hyper-responsiveness to stressful stimulation (Redei et al. 2001; Tejani-Butt et al. 2003). Exposing WKY rats to stress produces anhedonia, psychomotor

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retardation, ambivalence and negative memory bias on stressful events (Paré 1992, 1993, 1994a, 1996; Paré and Tejani 1996). Since norepinephrine (NE), serotonin (5-HT) and dopamine (DA) systems are involved in cognitive, emotional and motivational behaviors, we have previously reported that WKY rats exhibit significant differences in monoaminergic receptor densities in several limbic and motor brain nuclei compared to Wistar (WIS) rats (Tejani-Butt et al. 1994; Jiao et al. 2003, 2006; Yaroslavsky et al. 2006).

Preclinical and clinical studies support a role for the glutamatergic system in the pathology of depressive illness (Pittenger et al. 2007). The ionotropic glutamate N-methyl D-aspartate (NMDA) receptor subtype mediates excitatory amino acid synaptic transmission in the central nervous system (CNS). Abnormalities in NMDA receptor sites have been observed in postmortem brain tissue from patients with depression as well as suicide victims (Kim et al. 1982; Altamura et al. 1993; Nowak et al. 1995a; Berk et al. 2001; Law and Deakin 2001; Feyissa et al. 2009). Antidepressant drugs that bind to NMDA receptors modulate the release or reuptake of glutamate (Sills and Loo 1988; Bouron and Chatton 1999). Preclinical studies utilizing animal models have also implicated the glutamate system in depressive behavior (Paul 1997; Nowak et al. 1995b; Feyissa et al. 2009). Antidepressant drugs given after chronic mild stress produce changes in behavior and NMDA receptors, suggesting a direct effect of antidepressants on glutamate neurotransmission (Nowak et al. 1995b; Paul 1997).

Gamma (γ)-aminobutyric acid (GABA) is an inhibitory amino acid widely distributed within the CNS (Zachmann et al. 1966; Petroff 2002). GABA_A receptors are ionotropic, mostly postsynaptic and responsible for fast inhibitory postsynaptic potentials (Eder et al. 2001; Chang et al. 2003). Clinical studies have identified a GABA deficiency in the cerebrospinal fluid of patients with depression (Gold et al. 1980; Kasa et al. 1982). Animal models of depressive behavior such as the olfactory bulbectomized (OBX) rat model or the learned helpless model report reduced levels of GABA in several cortical and subcortical brain regions (Petty and Sherman 1981; Denis et al. 1993). Treatment with GABA_A receptor agonists reverses the depressive characteristics in these models (Petty and Sherman 1981; Lloyd et al. 1983; Borsini et al. 1987; Poncelet et al. 1987; Plaznik et al. 1988; Dennis et al. 1994). Tricyclic antidepressants and selective serotonin reuptake inhibitors produce changes in GABA_A receptor densities and sensitivity, further supporting the involvement of GABA_A receptor sites in the actions of antidepressant drugs (Suzdak and Gianutsos 1985; Barbaccia et al. 1986; Bagdy et al. 2000). Interestingly, low cortical GABA levels were reversed by antidepressant drugs that also reduced NMDA receptor sensitivity and/or transmission (Krystal et al. 2002; Paul and Skolnick 2003). Given the important role of the NMDA and GABA_A receptor systems in depressive illness, the present study mapped the distribution of NMDA and GABA_A receptor sites in cortical and subcortical brain regions of WKY rats using the technique of quantitative autoradiography.

Materials and Methods

All experimental protocols were reviewed and approved by the Perry Point VAH Institutional Review Committee for the use of animal subjects.

Animals and brain section preparation

Naïve male WKY (n=8) and WIS (n=8) rats, 290–340 grams, were used in this study. WIS rats were purchased from Harlan (Indianapolis, IN). WKY rats were raised in the Perry Point laboratory from the breeding stock initially obtained from Charles River Laboratories (Kingston, NY). Animals were individually housed at 22°C and placed on a 12-hr light/dark cycle, with lights on between 06:00 and 18:00 h. Animals were allowed to acclimate for two weeks prior to the sacrifice procedure. Rodent chow and water were kept available during the whole day. Animals were sacrificed by decapitation and the brains were removed immediately

and stored at -80°C till use. Brain tissue sections ($16\text{ }\mu\text{m}$) were cut at -18°C using a cryostat microtome according to the Brain Atlas of Paxinos and Watson (1998) and mounted on gelatin-coated microscope slides. Sections from plates 12, 30 and 42 were used in this study.

[^3H] MK-801 binding assay

NMDA receptors were labeled with [^3H] MK-801 (35Ci/mmol, Perkin Elmer) according to the method of Sakurai et al (1993) with minor modifications. The ligand concentration used in this study was based on previous K_d values reported for [^3H] MK-801 binding to NMDA receptors in various brain regions (Subramaniam et al. 1990; Sakurai et al. 1993). Preliminary experiments were performed in our laboratory using varying concentrations of [^3H] MK-801 (1–20 nM), and 15 nM ligand concentration was chosen to measure the binding of [^3H] MK-801 to NMDA receptors based on the high ratio of specific to non-specific binding. Brain sections were thawed and dried at room temperature, pre-washed in 50 mM Tris-HCl buffer (pH 7.4) for 30 min at 4°C . The washed sections were incubated in 50 mM Tris-HCl buffer containing 15 nM [^3H] MK-801 and 30 μM glutamate /10 μM glycine for 120 min at room temperature, rinsed for 30 min with cold 50 mM Tris-HCl buffer, dipped once in ice-cold distilled water, and immediately dried in a stream of cool air. Non-specific binding was measured in the presence of 50 μM non-radioactive MK-801. The slides were then dried at room temperature, transferred into cassettes, and exposed to Kodak BioMax MS film along with [^3H] standards. Following a 4-week exposure period at 4°C , the films were developed in Kodak GBX developer at room temperature.

[^3H] SR95531 binding assay

GABA_A receptors were labeled with [^3H] SR95531 (49.5Ci/mmol, Perkin Elmer) as previously described (Yao and Lawrence 2005). Preliminary experiments were performed in our laboratory to determine the appropriate concentration of [^3H] SR9551 given previously reported methods and K_d values (Yao and Lawrence 2005, Heaulme et al. 1987). Based on preliminary results, a ligand concentration of 6.5 nM was chosen to measure the binding of [^3H] SR9551 to GABA_A receptors based on the high ratio of specific to non-specific binding. Slides were warmed to room temperature followed by a 30 minute pre-incubation in Tris/Citrate buffer (50mM, pH- 7.4) containing 100 mM MgCl_2 . Sections were then dipped in cold buffer for 10 minutes and incubated in the same buffer solution containing [^3H] SR95531 (6.5 nM) for 45 minutes at 4°C . Non specific binding was determined with the addition of 10 mM GABA to the incubation buffer. The sections were then washed in ice cold phosphate buffer three times for five seconds each and distilled water two times for ten seconds each, and then dried under a gentle stream of cool air. Dry slides were transferred into cassettes and exposed to Kodak BioMax MS film along with [^3H] standards. The exposure time was 21 days for plate 12 and 28 days for plates 30 and 42. Films were developed using Kodak GBX developer.

Quantitation and statistics

The films were analyzed with ImageJ. Nonspecific binding was subtracted from the total binding to provide the specific binding in the regions of interest. The values of binding were expressed as mean $\pm$ S.E.M specific binding (fmol/mg brain protein). Statistical analysis was performed with Sigma Stat for Windows. The data in each region of interest were analyzed using a Student's t-test. The level of significance was set *a priori* at $p < 0.05$.

Representative autoradiograms of [^3H] MK-801 binding to NMDA receptors (A) and [^3H] SR95531 binding to GABA_A receptors (B) binding are shown in Fig. 1 for WKY rat (right) as well as for the comparison WIS (left) rat strain.

Results

The binding of [^3H] MK-801 to NMDA receptors and [^3H] SR95531 to GABA_A receptors in the anterior cingulate cortex

As shown in Table 1, WKY rats exhibited lower binding of [^3H] MK-801 to NMDA receptors in the anterior cingulate cortex (ACC) compared to WIS rats ($P < 0.001$). However, no significant difference in [^3H] SR95531 binding to GABA_A receptors was observed in the ACC between WKY and WIS rats (Table 2).

The binding of [^3H] MK-801 to NMDA receptors and [^3H] SR95531 to GABA_A receptors in the striatum

A significant strain difference in [^3H] MK-801 binding to NMDA receptor sites was observed in the caudate putamen (CPu) and the nucleus accumbens (NAc) (Table 1). Specifically, the binding of [^3H] MK-801 to NMDA receptors was significantly lower in WKY compared to WIS rats in the CPu and NAc ($P < 0.001$). The binding of [^3H] SR95531 to GABA_A receptors in the CPu was higher in WKY compared to WIS rats ($P < 0.05$; Table 2). However, no statistical differences were found in GABA_A receptor binding in the NAc between the two rat strains.

The binding of [^3H] MK-801 to NMDA receptors and [^3H] SR95531 to GABA_A receptors in the amygdala

Both NMDA and GABA_A receptor binding was measured in the amygdala. No significant strain difference in [^3H] MK-801 binding to NMDA receptor sites was observed in the amygdala (Table 1). In contrast, a higher binding of GABA_A receptor sites was found in the amygdala of WKY compared to WIS rats ($P < 0.05$; Table 2).

The binding of [^3H] MK-801 to NMDA receptors and [^3H] SR95531 to GABA_A receptors in the hippocampus

The binding of [^3H] MK-801 to NMDA receptors in WKY rats was significantly lower in the CA1 region of the hippocampus compared to WIS rats ($P < 0.001$, Table 1). However, no statistical strain difference was found in NMDA receptor binding in the dentate gyrus (DG), CA2, CA3 subregion of the hippocampus between the two rat strains. In contrast, the specific binding of [^3H] SR95531 to GABA_A receptor sites was significantly increased in the DG ($P < 0.05$), CA2 ($P < 0.05$) and CA3 ($P < 0.05$) regions of the hippocampus of WKY compared to WIS rats, while no differences in binding were observed in the CA1 region (Table 2).

The binding of [^3H] MK-801 to NMDA receptors and [^3H] SR95531 to GABA_A receptors in the midbrain regions

Within the mesencephalon, NMDA and GABA_A receptor binding was observed in the periaqueductal grey (PAG), ventral tegmental area (VTA) and substantia nigra pars reticulata (SNr) regions. While the binding of [^3H] MK-801 to NMDA receptors showed no differences in the PAG and VTA, the binding was lower in the SNr of WKY when compared to WIS rats ($P < 0.05$, Table 1). However, the binding of [^3H] SR95531 to GABA_A receptors was greater in the PAG ($P < 0.05$) and SNr ($P < 0.05$) regions, with no differences measured in the VTA of WKY when compared to WIS rats (Table 2).

Discussion

Strain differences in NMDA receptor sites

In WKY rats, a lower binding of [^3H] MK-801 to NMDA receptor sites was measured in several key brain structures that are involved in cognition, movement, memory and reward when compared to WIS rats. In rats and monkeys, the anatomical projections between the cerebral

cortex and subcortical areas, including the CPu, NAc, VTA and hippocampus are formed predominantly from excitatory projections (Ongür et al. 2000, 2003; Drevets and Price 2008). Our current findings are similar to previous reports of decreased NMDA receptor binding in several cortical and subcortical regions in the OBX rat model of depressive behavior (Robichaud et al. 2001; Song et al. 2005). Lower binding of [³H] MK-801 to NMDA receptors in WKY rats may reflect a reduced number of functional receptors or a deficiency in the NMDA receptor complex.

Anatomically, the ACC has prominent connections with limbic structures and visceral control structures such as the hypothalamus and PAG (Ongür et al. 2000). The ACC is thought to play a key role in processing attention as well as emotional information, and is involved in the pathogenesis of depression (Auer et al. 2000; Elliott et al. 2002; Mayberg 1999; Pfleiderer et al. 2003). A disturbance in glutamatergic pyramidal neurons expressing NMDA receptors in the ACC has been found in depressed patients (Akbarian et al. 1996; Cotter et al. 2001; Rajkowska et al. 1999). Since dysfunctions in these circuits can produce depressive symptoms (Drevets et al. 2004), it is reasonable to speculate that lower NMDA receptor binding in the ACC of WKY rats may reflect abnormalities in the excitatory glutamatergic system in this rat strain.

WKY rats show lower binding of [³H] MK-801 to NMDA receptors in the NAc and CA1 region of the hippocampus compared to WIS rats. These two structures are part of the limbic system associated with motivational activity and memory functions, and drives alcohol as well as other drug dependence (Miller 1991). Thus, lower NMDA receptor binding in the NAc and CA1 regions in WKY rats may reflect a dysfunction in glutamatergic receptors in these limbic nuclei, leading to psychomotor retardation (Paré 1994a), anhedonia (Paré 1994b, 2000) and a preference for ethanol drinking behavior (Jiao et al. 2006). Since glutamatergic projections to limbic areas also regulate DA and GABA systems (Chergui et al. 1993; Wang and French 1993; Jedema and Moghaddam 1996), it is possible that NMDA receptor-mediated regulation of DA and GABA neurotransmission may be compromised, contributing to the noted depressive-like behaviors described in this rat strain (Jiao et al. 2003).

The binding of [³H] MK-801 to NMDA receptors was reduced in the CPu and SNr of WKY compared to WIS rats. These two structures make up the nigrostriatal pathway which is involved in motor control. Cellular, electrophysiological and neurochemical investigations have shown that NMDA receptors interact with DA type-1 (D1) receptors, located on nigrostriatal medium spiny output neurons, leading to a facilitating action on locomotor activity (Cepeda and Levine 1998; Schoffelmeer et al 2000; Marti et al 2002; David et al. 2005). A reduction in [³H] MK-801 binding to NMDA receptors may decrease D1-like receptor activation, thereby decreasing locomotor activity. In the present study, lower NMDA receptor binding observed in the nigrostriatal pathway may be partly responsible for the reduced locomotor activity previously reported in the WKY rat strain (Paré 1994a).

Strain differences in GABA_A receptor sites

In contrast to the lower binding of [³H] MK-801 to NMDA receptors in WKY rats, a significantly higher binding of [³H] SR95531 to GABA_A receptors was measured in similar brain regions of WKY rats when compared to the control WIS rat strain. The hippocampus is densely packed with GABAergic neurons and plays an important role in memory formation associated with depressive behaviors (Banks et al. 2000). A higher binding [³H] SR95531 to GABA_A receptors was found in the CA2, CA3 and DG regions of the hippocampus of WKY compared to WIS rats. Higher GABA_A receptor binding may reflect a compensatory response to abnormally low GABA levels in these brain regions. In agreement with our results, increased GABA_A receptor binding was reported in the hippocampus in the OBX rat model of depressive behavior (Dennis et al., 1993). In addition, a reduction in GABA levels in the hippocampus

was shown to be associated with helpless behavior in animals (Sherman and Petty 1982) and GABA levels were reduced following a forced swim test in mice (Briones-Arana et al. 2005). In light of these studies, the WKY rat strain, which has previously demonstrated memory bias as well as other characteristics of depressive behavior, may also have an inherent deficit in GABA levels in several regions of the hippocampus (Paré 1996).

The amygdala is involved in anxiety and aversive memory formation (Davis and Whalen 2001; Chhatwal et al. 2005). While stressful stimuli reduce GABA levels (Stork et al. 2002), GABA_A agonists injected into the amygdala reduce anxiety-like behaviors (Beuno et al. 2005). An increase in GABA turnover was measured in the amygdala of OBX rats, suggesting a possible reduction in extracellular GABA levels (Janscár and Leonard 1984). These studies indicate that GABA levels in the amygdala are negatively correlated with anxious behaviors. Previous studies have described the WKY rat as being hyper responsive to stressful stimuli under several experimental conditions (Tejani-Butt et al. 1994). Therefore, the higher binding of [³H] SR95531 to GABA_A receptors in the WKY rat strain may reflect reduced GABAergic neurotransmission under naïve conditions and may predispose these animals to anxiety like behaviors when challenged with a stressful situation. Since a similar increase in GABA_A receptor binding was also seen in the PAG, a region involved in anxiety and implicated in the pathophysiology of depression (Nauta and Domesick 1984), it further supports the stress-sensitive phenotype of the WKY rat strain.

The majority of afferent projections to the SNr are GABAergic and they arise from various regions including the CPu (Tepper and Lee 2007). As mentioned in the previous section, DA neurons originating in the SN, specifically the pars compacta (SNc) region, project to the CPu as part of the nigrostriatal DA pathway involved in motor control. Within the SNr, GABA_A receptors are located on GABAergic neurons, and upon activation, inhibit neuronal firing of local GABA neurons, some of which project to the SNc to influence DA release (Tepper 1995). Thus, dysfunction of GABAergic system in this circuit may lead to abnormal activity and/or motor abnormalities. Our finding of increased GABA_A receptor binding in the CPu and SNr may provide a neurochemical explanation for the previously reported reduction in locomotor activity in WKY compared to WIS rats (Paré 1994a).

Implications of NMDA and GABA_A receptor sites in depressive behavior

Converging evidence suggest that abnormalities in both glutamate and GABA neurotransmitter systems may contribute to the pathophysiology of depression (Krystal et al. 2002; Cryan and Kaupmann 2005). Sanacora and colleagues reported that alterations in cortical GABA and glutamate levels were particularly associated with melancholic and psychotic features in major depression disorder (MDD), further suggesting that MDD coexists with abnormalities in major excitatory and inhibitory neurotransmitter systems (Sanacora et al. 2004). It was hypothesized that a single alteration in the major excitatory or inhibitory neurotransmitter systems may lead to opposite alterations (Sanacora et al. 2004) because the metabolic pathways regulating the synthesis and cycling of GABA and glutamate are tightly coupled (Erecinska et al. 1996; Sepkuty et al. 2002; Patel et al. 2001).

Interestingly, we have found a negative correlation between the binding of [³H] MK-801 to NMDA receptors and [³H] SR95531 binding to GABA_A receptors in several brain regions examined in this study. The binding of [³H] MK-801 to NMDA receptors was significantly lower in several cortical and subcortical regions, while the binding of [³H] SR95531 to GABA_A receptors was significantly higher in similar regions of WKY compared to WIS rats. Based on these differences in receptor binding, we can speculate that an overactive NMDA receptor system coupled with a hypoactive GABA_A receptor system may be involved in producing the depressive and stress sensitive phenotype in the WKY rat strain. At the present time, it is not known whether GABA_A receptor upregulation plays a primary role in the

depressive-like behaviors noted in WKY rats, or whether it represents a compensatory response to GABA deficiency or glutamate hyper-excitability. However, the negative correlation observed between NMDA and GABA_A receptor binding may reflect a dysregulation in the glutamate-GABA neurotransmission system in WKY rats.

Conclusion

In summary, a significant strain difference exists in the binding of [³H] MK-801 to NMDA receptors and [³H] SR95531 binding to GABA_A receptors between WKY and WIS rats in several cortical and subcortical brain regions. Over all, the binding of [³H] MK-801 to NMDA receptors was significantly lower in most brain regions measured in WKY rats. In contrast, the binding of [³H] SR95531 to GABA_A receptors was significantly higher in similar brain areas in WKY compared to WIS rats. Given that these two rat strains differ in their behavioural, endocrine and neurochemical profile, the observed strain differences in NMDA and GABA_A receptor binding suggest that these two neurotransmitters systems may be contributing to the depressive and stress sensitive phenotype of the WKY rat strain.

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Figure 1A

Figure 1B

Fig. 1. Representative autoradiographs from Plates 12, 30 and 42 showing total [^3H] MK-801 binding to NMDA receptors (A) and [^3H] SR95531 binding to GABA_A receptors (B) in WKY rat (right) and comparison WIS rat (left) brains.

Table 1The binding of [³H] MK-801 to NMDA receptors in WIS and WKY rat brain

Regions of interest	WIS	WKY	P Values
Cingulate cortex	106.0 ± 5.4	60.1 ± 1.9	<0.001 **
Striatum			
Caudate putamen	40.1 ± 3.2	17.2 ± 1.1	<0.001 **
Nucleus accumbens	45 ± 5.2	21.6 ± 1.5	0.001 **
Amygdala	26.3 ± 3.0	28 ± 2.0	0.637
Hippocampus			
CA1	82.9 ± 3.4	57.9 ± 4.4	< 0.001 **
CA2	77.2 ± 3.2	79.7 ± 2.2	0.54
CA3	61.3 ± 3.7	58.6 ± 1.9	0.383
Dentate gyrus	52.6 ± 4.6	61.4 ± 3.9	0.287
Midbrain regions			
Pariaqueductal grey	11.6 ± 0.98	9.3 ± 1.3	0.183
Ventral tegmental area	16.6 ± 1.8	12.9 ± 0.87	0.180
Substantia nigra	18.6 ± 2.2	12.7 ± 0.59	0.026 *

Values are mean ± SEM in fmol/mg protein.

* p < 0.05,

** p < 0.001 for WKY versus WIS

Table 2The binding of [³H] SR95531 to GABAA receptors in WIS and WKY rat brain

Regions of interest	WIS	WKY	P Values
Cingulate cortex	2.49±0.74	2.73±0.49	0.801
Striatum			
Caudate putamen	0.68± 0.22	1.65±0.3	0.030*
Nucleus accumbens	1.08±0.39	1.43±0.36	0.534
Amygdala	35.2±2.1	49.6 ± 4.2	0.012*
Hippocampus			
CA1	60.3±3.47	66.9±5.9	0.355
CA2	43.2±4.16	59.3±5.27	0.037*
CA3	42.7±3.28	66.6±8.3	0.024*
Dentate gyrus	51.9±2.64	74.8±6.65	0.007*
Midbrain regions			
Pariaqueductal grey	14.1±1.44	22.9±2.63	0.016*
Ventral tegmental area	13.7±1.09	16.3±1.04	0.141
Substantia nigra	14.6± 1.10	20.7±2.43	0.032*

Values are mean ± SEM in fmol/mg protein.

*
p < 0.05.**
p<0.001 for WKY versus WIS