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Effects of Age and Gender on the Expression of Brain-Derived Neurotrophic Factor mRNA in Rat Retrosplenial Cortex Following Administration of Dizocilpine

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Using in situ hybridization, we studied the effects of age and gender on the expression of brain-derived neurotrophic factor (BDNF) mRNA and heat shock protein hsp-70 mRNA in the rat retrosplenial cortex following administration of the noncompetitive NMDA receptor antagonist (+)-MK-801 (dizocilpine). Male and female Sprague–Dawley rats (5 weeks, 12 weeks, or 10 months old) were given a single intraperitoneal injection of saline (1 ml/kg) or dizocilpine (0.3, 1.0, or 3.0 mg/kg). No expression of BDNF mRNA and hsp-70 mRNA was detected in the rat retrosplenial cortex after administration of saline (1 ml/kg, IP). Administration of dizocilpine (0.3, 1.0, or 3.0 mg/kg, IP) caused a marked induction of BDNF mRNA and hsp-70 mRNA in the retrosplenial cortex of male and female rats, in a dose-dependent manner. Female rats were more sensitive to the induction of BDNF mRNA and hsp-70 mRNA in the retrosplenial cortex by dizocilpine as compared to male rats. It was also found that adult (12 weeks old) and aged (10 months old) rats were more sensitive to the induction of hsp-70 mRNA and BDNF mRNA in the retrosplenial cortex by dizocilpine as compared to young (5 weeks old) rats. These results suggest that the age and gender differences observed in the expression of BDNF mRNA and hsp-70 mRNA in the retrosplenial cortex by dizocilpine may be associated with the differences in dizocilpine-induced neurotoxicity observed with gender and age within the same region.
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KEY WORDS: *NMDA receptor antagonist; Brain-derived neurotrophic factor; Heat shock protein; Aging; Gender; Retrosplenial cortex*

Several observations suggest that altered glutamatergic neurotransmission may play a role in the etiology and pathophysiology of schizophrenia (reviews by Javitt and Zukin 1991; Olney and Farber 1995; Coyle 1996; Tamminga 1998). Phencyclidine (PCP) and its analog ketamine have psychotomimetic properties in healthy human subjects and have been shown to cause a prolonged worsening of symptoms in stabilized chronic schizophrenic patients (Luby et al. 1959; Krystal et al. 1994). PCP and ketamine are noncompetitive antagonists of the ion-channel of the N-methyl-D-aspartate (NMDA)

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subtype of glutamate receptor, and it is known that the psychotomimetic properties of PCP and ketamine are attributable to noncompetitive blockade of the NMDA receptor (Javitt and Zukin 1991). Ketamine is still used as an anesthetic in humans, and it is known that the ability of ketamine to induce psychosis is age dependent, with susceptibility to these effects not occurring in humans until adolescence and peak sensitivity occurring in early adulthood (Reich and Silvay 1989). In addition, it has also been suggested that PCP-induced psychosis may have a similar age-dependency profile (Baldridge and Bessen 1990; Welch and Correa 1980).

Noncompetitive NMDA receptor antagonists such as PCP, (+)-MK-801 (dizocilpine), and ketamine have been reported to cause neurotoxicity in the retrosplenial cortex of rat brain after administration of a single dose (Olney et al. 1989). It has been shown that dizocilpine causes reversible pathological changes (neuronal vacuolization) in retrosplenial cortical neurons at doses of 2.5 mg/kg or less and that, at a dose of 5 mg/kg, dizocilpine induces irreversible necrosis of these neurons (Olney and Farber 1995). Furthermore, it has been reported that vulnerability to dizocilpine-induced neurotoxicity in the rat retrosplenial cortex is age dependent, with the onset of sensitivity being approximately at puberty (45 days of age) and becoming maximal in early adulthood (Farber et al. 1995). Thus, rats display a gradual onset of susceptibility to NMDA receptor antagonist-induced neuronal injury in late adolescence just as humans become susceptible to ketamine (or PCP)-induced psychosis and to the symptomatic expression of schizophrenia in late adolescence (Olney and Farber 1995). Moreover, it has been shown that female rats are more sensitive to the neurotoxicity of NMDA receptor antagonists than male rats (Fix et al. 1995; Auer 1996). However, the precise mechanism underlying the neurotoxicity of NMDA receptor antagonists in rat retrosplenial cortex is currently unclear (reviews by Ellison 1995; Olney and Farber 1995; Farber et al. 1998).

It has been shown that 70 kDa heat shock protein HSP-70, which is not normally present in the rat brain, is induced within vulnerable brain cells in response to a number of different manipulations that induce cellular injury paradigms (reviews by Brown 1994; Sharp and Sagar 1994; Massa et al. 1996). Furthermore, it has been reported that heat shock protein HSP-70, which is known as a sensitive marker of reversible neuronal injury, is induced in the retrosplenial cortex after administration of PCP, dizocilpine, or ketamine (Sharp et al. 1991; Sharp and Sagar 1994; Hashimoto et al. 1996; 1997). Heat shock proteins play an important role in cellular repair and protective mechanisms (reviews by Brown 1994; Sharp and Sagar 1994; Massa et al. 1996), thus the expression of heat shock protein HSP 70 may be a compensatory response of these neurons to the neurotoxicity produced by NMDA receptor antagonists.

Brain-derived neurotrophic factor (BDNF) is the most abundant and widely expressed member of the neurotrophin family within the brain. It has been previously demonstrated that BDNF promotes the survival and differentiation of a broad variety of central nervous system neurons (reviews by Lindsay et al. 1994; Lindvall et al. 1994). Experimental manipulations that induce seizures have been shown to induce widespread dramatic elevations in BDNF mRNA levels in areas of the brain including the hippocampus, the piriform, and entorhinal cortex and neocortex (Ernfors et al. 1991; Isackson et al. 1991; Zafra et al. 1990; Hashimoto et al. 1998b). It has also been demonstrated that administration of PCP or dizocilpine causes a marked increase in BDNF mRNA levels in the rat retrosplenial cortex, an area that is most vulnerable to NMDA receptor antagonist-induced neurotoxicity (Hashimoto et al. 1998a; Castren et al. 1993; Hughes et al. 1993). Based on these findings, it has been suggested that expression of BDNF may occur as a trophic response to neuronal injury.

The aforementioned findings suggest that susceptibility to the neurotoxicity induced by NMDA receptor antagonists is dependent on the age and gender of rats and that expression of heat shock protein HSP-70 and BDNF may play a role in neuronal injury induced by NMDA receptor antagonists. Therefore, the present study was undertaken to study the effects of age and gender on the expression of BDNF mRNA and hsp-70 mRNA in the rat retrosplenial cortex following administration of dizocilpine.

METHODS

Animals

Male and female Sprague–Dawley rats (Nihon Clea, Tokyo, Japan, 5 or 12 weeks old and 10 months old) were housed separately under a 12-h light/12-h dark cycle with free access to food and water. All experiments were carried out in accordance with the *NCNP Guide for the Care and Use of Laboratory Animals.*

In Situ Hybridization

Groups of animals ($n = 4$) were injected intraperitoneally with vehicle (saline; 1 ml/kg) or dizocilpine (0.3, 1.0, or 3.0 mg/kg as the salt, Research Biochemicals Inc., Natick, MA). Four hours after administration, animals were narcotized with carbon dioxide, and the brains were removed and frozen. Twenty μm-thick frozen coronal sections were cut on a Bright cryostat and thaw-mounted onto silanized slides (Dako, Japan). Sections were frozen at −80°C until use. In situ hybridization of hsp-70 mRNA and BDNF mRNA was performed by the method previously described by Hashimoto et al. (1997, 1998a,b). The oligonucleotide

(30 mer) used for in situ hybridization of hsp-70 mRNA was 5′-CGATCTCCTTCATCTTGGTCAGCACCATGG-3′ complementary to bp 122 to 129 of rat hsp-70. The oligonucleotide (48 mer) used for in situ hybridization of BDNF mRNA was 5′-CAGTTGGCCTTTTGATAC CGGGACTTTCTCCAGGACTGTGACCGTCCC-3′ complementary to bp 562-609 of rat BDNF. Control sections were hybridized with a sense oligonucleotide probe and showed no evidence of specific hybridization. The oligonucleotide probe was labeled at the 3′ end with [^{35}S]dATP (> 30 TBq/mmol, Amersham, UK) using an oligonucleotide 3′-end labeling system (DuPont/New England Nuclear, MA), and purified over NENSORB™ 20 cartridge (DuPont/New England Nuclear, MA). The sections were fixed in 4% paraformaldehyde–0.1 M phosphate buffer for 30 min at 4°C, rinsed twice in 0.1 M phosphate buffered saline (PBS), acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0), rinsed twice in 2 × SSC (0.15 M NaCl, 15 mM sodium citrate), then dehydrated and delipidated through a graded series of ethanol and chloroform. After being air dried, the sections were hybridized overnight at 42°C with 0.5–1 × 10^6 cpm of the labeled probe in 100 μl of the hybridization solution (Oncor, Inc., Gaithersburg, MD) including 0.1 M dithiothreitol. After hybridization, each slide was washed twice for 5 seconds in 1 × SSC at 55°C four times for 15 min in 1 × SSC at 55°C, for 1 h in 1 × SSC at room temperature and twice in deionized water for 5 min. Sections were dipped in 60, 80, 90, 95, and 100% ethanol and air dried. The slides were exposed to Hyperfilm βmax (Amersham, UK) for 2 to 3 weeks before being developed. Although the posteriormost portion of cingulate cortex is given different designations in various anatomic references, it is referred to here as the retrosplenial cortex according to Paxinos and Watson (1997).

Statistical Analysis

Densitometric analysis of sections was carried out using Macintosh-based image analysis software (NIH image). The data were initially analyzed using a three-way analysis of variance (ANOVA) (age × gender × treatment). Where a significant interaction in the between-subjects variables (age, gender, treatment) was found, a subsequent two-way ANOVA was performed. Furthermore, where a significant interaction for the two-way ANOVA was found, a subsequent one-way ANOVA was carried out, followed by a post-hoc comparison using Scheffe's test. The criterion of significance was $p < .01$.

RESULTS

No expression of BDNF mRNA and hsp-70 mRNA was detected in the rat retrosplenial cortex after administration of saline (1 ml/kg, IP). Administration of dizocilpine (0.3, 1.0, or 3.0 mg/kg, IP) caused a marked induction of hsp-70 mRNA and BDNF mRNA in the retrosplenial cortex of male and female rats, in a dose-dependent manner (Figures 1–3). Female rats were more sensitive to the induction of BDNF mRNA and hsp-70 mRNA in the retrosplenial cortex by dizocilpine than male rats. The induction of BDNF mRNA and hsp-70 mRNA in the retrosplenial cortex of adult (12 weeks old) and aged rats (10 months old) by dizocilpine was greater than in the young (5 weeks old) rats.

Effects of Age and Gender on Dizocilpine-Induced BDNF mRNA Expression

The three-way ANOVA indicated significant effects of age, gender, and treatment on BDNF mRNA expression (age: $F[2,72] = 113.8$, $p < .001$; gender: $F[1,72] = 20.6$, $p < .001$; treatment: $F[3,72] = 134.8$, $p < .001$) with significant interactions (age × treatment: $F[6,72] = 17.9$, $p < .001$; gender × treatment: $F[3,72] = 23.0$, $p < .001$). The two-way ANOVA on the young groups demonstrated a significant effect of treatment ($F[3,24] = 5.7$, $p < .004$) without a significant interaction. However, the subsequent one-way ANOVAs did not show a significant effect of treatment for either the young female group ($F[3,12] = 2.624$, $p = .09$) or the young male group ($F[3,12] = 4.07$, $p = .03$). The two-way ANOVA on the adult groups demonstrated a significant effect of treatment ($F[3,24] = 78.0$, $p < .0001$) with a significant interaction (gender × treatment: $F[3,24] = 15.1$, $p < .0001$). The subsequent one-way ANOVA on the adult female group indicated a significant effect of treatment ($F[3,12] = 29.9$, $p < .0001$) and the post-hoc comparison using Scheffe's test demonstrated that dizocilpine had significant treatment effects at 0.3 mg/kg ($p = .0001$), 1.0 mg/kg ($p < .0001$) and 3.0 mg/kg ($p < .001$). The one-way ANOVA on the adult male group also showed a significant effect of treatment ($F[3,12] = 77.8$, $p < .0001$), and the post-hoc comparison using Scheffe's test demonstrated that dizocilpine has significant treatment effects only at 1.0 mg/kg ($p < .0001$) and 3.0 mg/kg ($p < .0001$), but not 0.3 mg/kg. The two-way ANOVA on the aged groups demonstrated a significant effect of treatment ($F[3,24] = 78.0$, $p < .0001$) with a significant interaction (gender × treatment: $F[3,24] = 15.4$, $p < .0001$). The subsequent one-way ANOVA on the aged female group showed a significant treatment effect ($F[3,12] = 33.8$, $p < .0001$) and the post-hoc comparison using Scheffe's test indicated that dizocilpine had significant treatment effects at 0.3 mg/kg ($p = .0001$), 1.0 mg/kg ($p = .0001$) and 3.0 mg/kg ($p < .0001$). The one-way ANOVA on the aged male group also demonstrated that dizocilpine had significant treatment effects, but post-hoc comparison with Scheffe's test revealed signif-

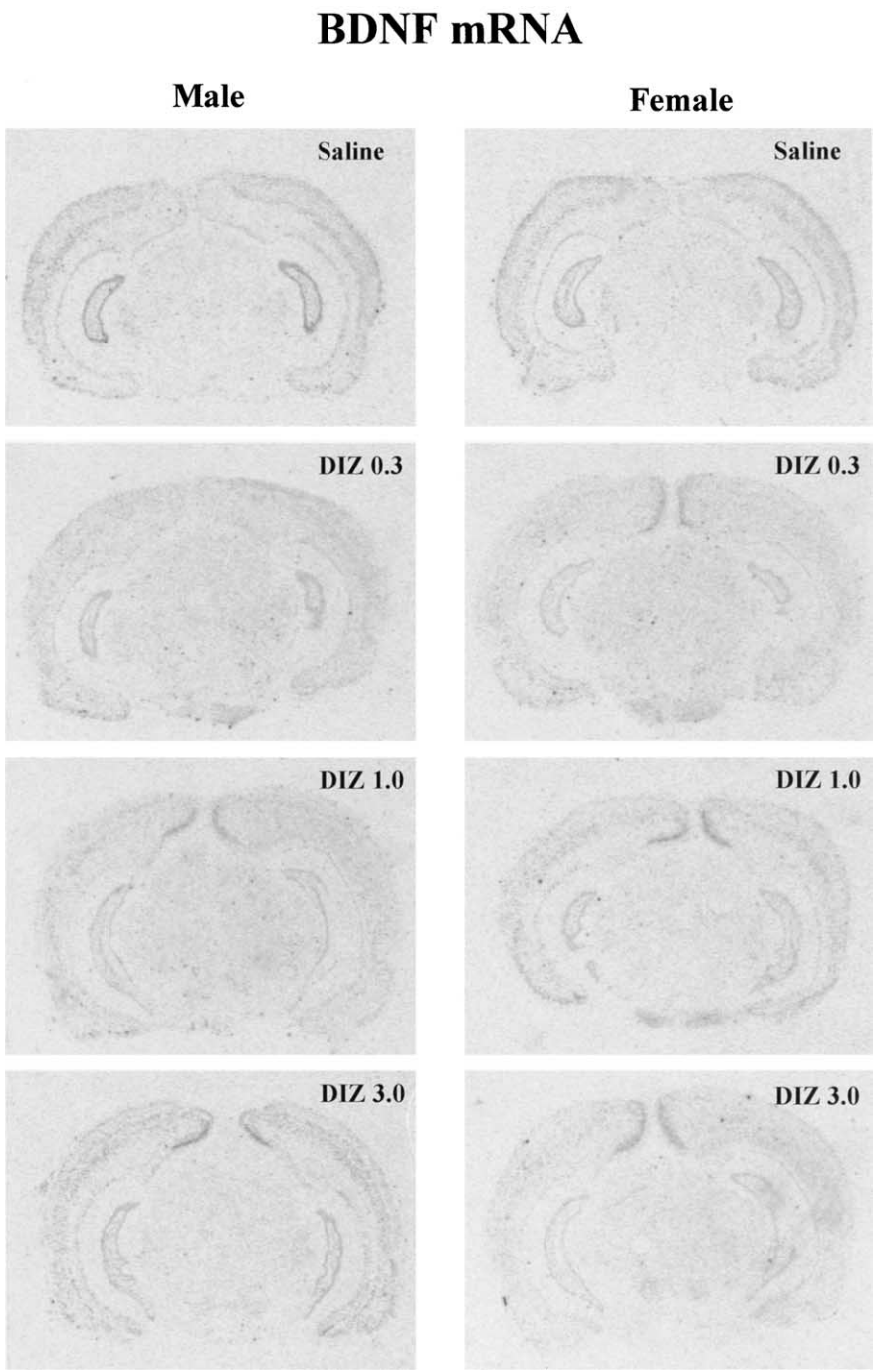

Figure 1. Expression of BDNF mRNA in the rat brain induced by administration of dizocilpine. Male and female SD rats (12 weeks olds) were injected intraperitoneally with saline (1 ml/kg) or dizocilpine (0.3, 1.0, or 3.0 mg/kg). Animals were killed 4 hours after treatment for in situ hybridization.

icance only at 1.0 mg/kg ($p < .0001$) and 3.0 mg/kg ($p < .0001$), but not 0.3 mg/kg.

Effects of Age and Gender on Dizocilpine-Induced hsp 70 mRNA Expression

The three-way ANOVA indicated significant effects of age, gender, and treatment on hsp70 mRNA expression (age: F [2,72] = 69.3, $p < .0001$; gender: F [1,72] = 15.6, $p = .0002$; treatment: F [3,72] = 96.1, $p < .0001$) with significant interactions (age × treatment: F [6,72] = 10.6, $p < .0001$; gender × treatment: F [3,72] = 15.3, $p < .0001$). The two-way ANOVA on the young groups demonstrated a significant effect of treatment (F [3,24] = 6.5, $p = .002$) without a significant interaction. However, the subsequent one-way ANOVA did not show

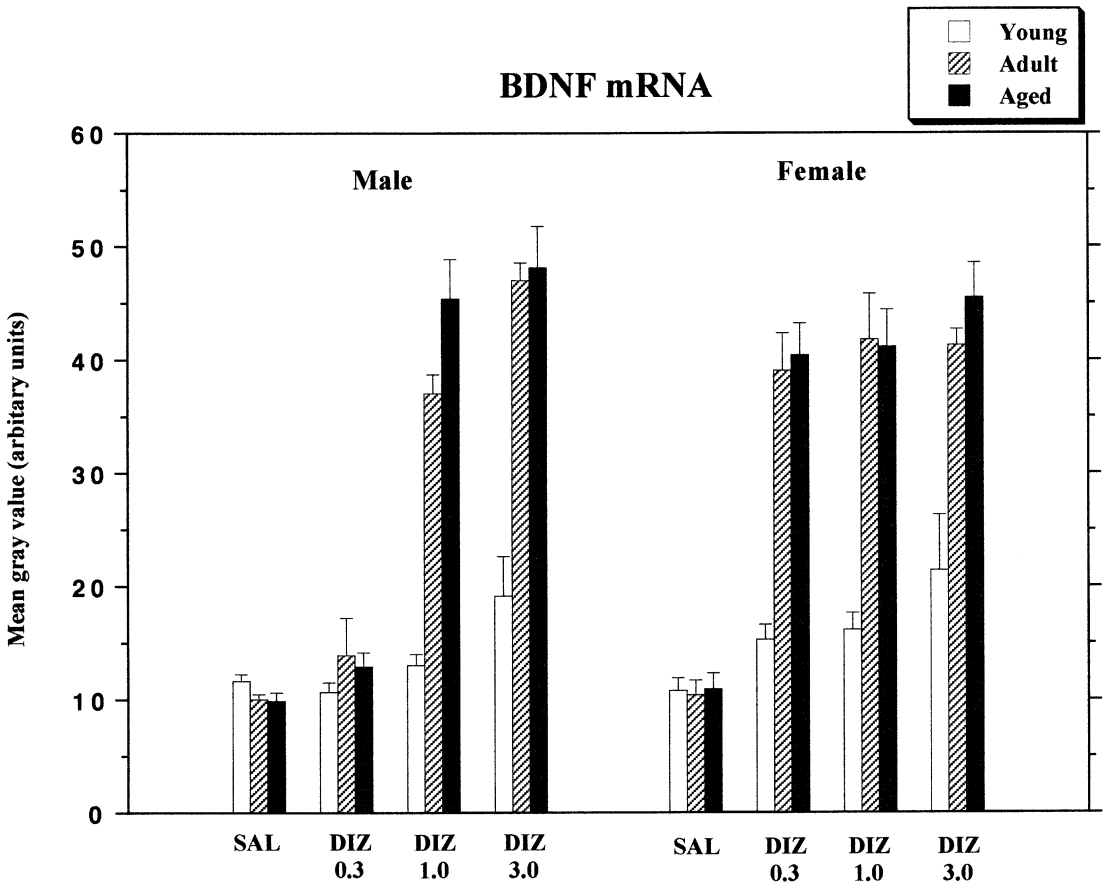

Figure 2. Expression of BDNF mRNA in the rat retrosplenial cortex induced by administration of dizocilpine. Male and female SD rats (5 weeks, 12 weeks, or 10 months olds) were injected intraperitoneally with saline (1 ml/kg) or dizocilpine (0.3, 1.0, or 3.0 mg/kg). Animals were killed 4 hours after treatment for in situ hybridization. Densitometric analysis of sections was performed using Macintosh-based image analysis software (NIH image) as described in Methods. The values are the mean ± SEM of four rats.

significant treatment effects in either the young female group ($F [3,12] = 2.8$, $p = .08$) or the young male group ($F [3,12] = 4.0$, $p = .03$). The two-way ANOVA on the adult groups demonstrated significant effects of treatment and gender (gender: $F [1,24] = 27.0$, $p < .0001$; treatment: $F [3,24] = 90.4$, $p < .0001$) with a significant interaction (gender × treatment: $F [3,24] = 15.1$, $p < .0001$). The subsequent one-way ANOVA on the adult female group indicated significance ($F [3,12] = 41.5$, $p < .0001$), and the post-hoc comparison using Scheffe's test demonstrated that dizocilpine had significant treatment effects at 0.3 mg/kg ($p < .0001$), 1.0 mg/kg ($p < .0001$) and 3.0 mg/kg ($p < .0001$). The one-way ANOVA on the adult male group also showed significant treatment effects ($F [3,12] = 65.9$, $p < .0001$), but the post-hoc comparison using Scheffe's test demonstrated that dizocilpine had significant treatment effects only at 1.0 mg/kg ($p < .0001$) and 3.0 mg/kg ($p < .0001$) but not 0.3 mg/kg. The two-way ANOVA on the aged groups demonstrated a significant effect of treatment ($F [3,24] = 64.0$, $p < .0001$) with a significant interaction (gender × treatment: $F [3,24] = 18.8$, $p < .0001$). The subsequent one-way ANOVA on the aged female group showed a significant treatment effect ($F [3,12] = 22.9$, $p < .0001$), and the post-hoc comparison using Scheffe's test indicated that dizocilpine had significant treatment effects at 0.3 mg/kg ($p = .0001$), 1.0 mg/kg ($p = .0004$), and 3.0 mg/kg ($p = .0002$). The one-way ANOVA on the aged male group also demonstrated a significant effect of treatment, but the post-hoc comparison using Scheffe's test showed that dizocilpine has significant treatment effects only at 1.0 mg/kg ($p < .0001$) and 3.0 mg/kg ($p < .0001$) but not 0.3 mg/kg.

DISCUSSION

The major finding of the present study is that the marked expression of BDNF mRNA and hsp-70 mRNA in rat retrosplenial cortex induced by administration of dizocilpine is dependent upon the age and gender of the animal used. As mentioned in the introduction, the

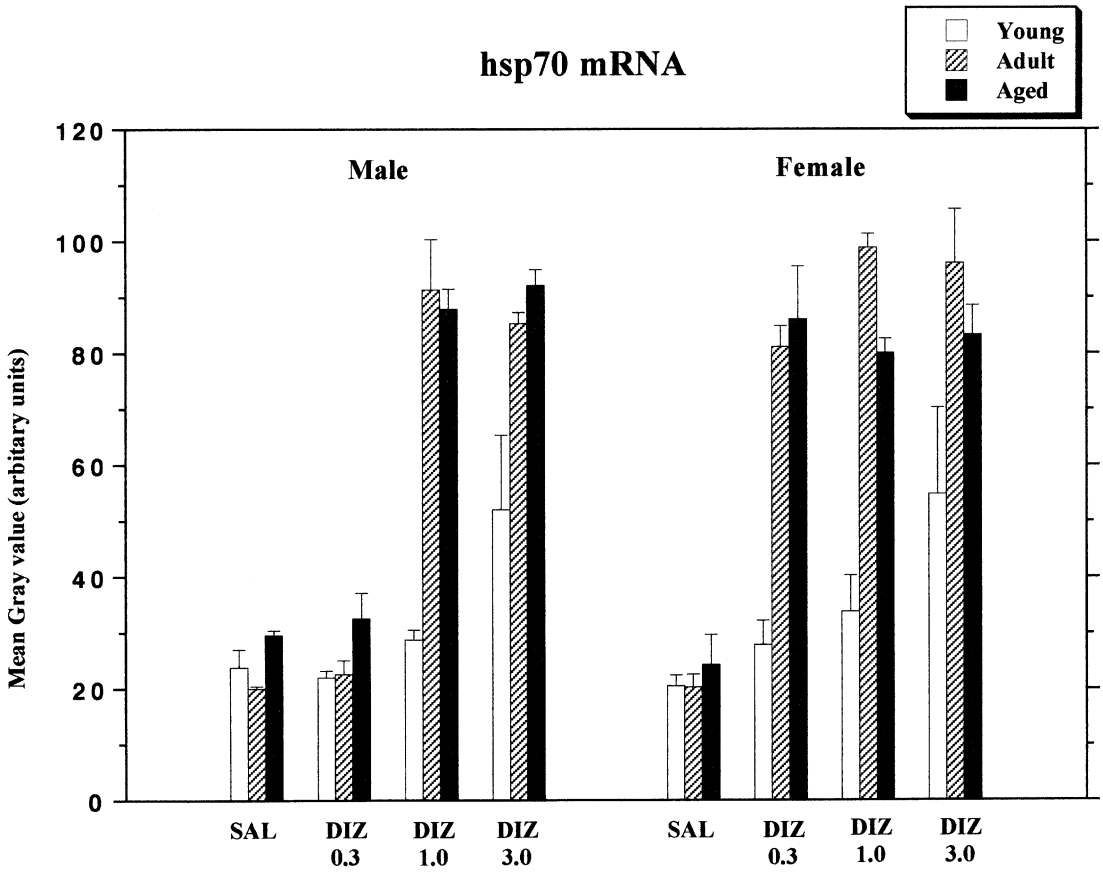

Figure 3. Expression of hsp-70 mRNA in the rat retrosplenial cortex induced by administration of dizocilpine. Male and female SD rats (5 weeks, 12 weeks, or 10 months olds) were injected intraperitoneally with saline (1 ml/kg) or dizocilpine (0.3, 1.0, or 3.0 mg/kg). Animals were killed 4 hours after treatment for in situ hybridization. Densitometric analysis of sections was performed using Macintosh-based image analysis software (NIH image) as described in Methods. The values are the mean ± SEM of four rats.

expression of BDNF mRNA and hsp-70 mRNA may occur as a compensatory response and/or trophic response to neuronal injury, such as dizocilpine-induced neurotoxicity. Therefore, it is likely that the age and gender differences in the expression of hsp-70 mRNA and BDNF mRNA in rat retrosplenial cortex induced by administration of dizocilpine may reflect the differences associated with dizocilpine-induced neurotoxicity in the retrosplenial cortex, which is also dependent upon the age and gender of animals used (Farber et al. 1995; Fix et al. 1995; Auer 1996).

Specific reasons for the difference in expression of BDNF mRNA and hsp-70 mRNA induced by dizocilpine with age are currently unknown, but the results are consistent with previous reports of dizocilpine-induced neurotoxicity in rat retrosplenial cortex being age dependent (Farber et al. 1995; Fix et al. 1995; Auer 1996). In female rats, susceptibility to dizocilpine-induced neurotoxicity was first detected at 6 weeks, which is when the first estrus cycle (menarche) occurs, with susceptibility steadily increasing with age between 6 and 12 weeks (roughly comparable to adolescence) before reaching a plateau in adulthood between 12 and 16 weeks, which is when maximal fertility (frequency of ovulation and size of brood) is finally attained (Farber et al. 1995). It is also known that susceptibility to the psychotomimetic effects of ketamine is minimal or absent in children and becomes maximal in early adulthood (White et al. 1982; Reich and Silvay 1989). As described by Farber et al. (1995), it seems that the age-dependency for NMDA receptor antagonist-induced neurotoxicity in rat brain roughly parallels the age dependency for sensitivity to ketamine-induced psychosis and for the emergence of the symptoms of schizophrenia in humans, although age comparisons between humans and rats are inherently problematic.

Shimada et al. (1997) reported that high-affinity [^{3}H]dizocilpine binding showed an increase during development (8 vs. 2 months) in rat hippocampus but that no senescence-related changes (25 vs. 8 months) were observed in rat brain. In our study, the age difference between young (5 weeks) rats and adult (12 weeks) or

aged (10 months) rats was shown. Therefore, it is likely that a difference in the density of NMDA receptor expression may, in part, contribute to the age-dependent expression of BDNF mRNA and hsp-70 mRNA in rat retrosplenial cortex by dizocilpine.

As previously stated, specific reasons for the gender differences in the expression of BDNF mRNA and hsp-70 mRNA by dizocilpine are also currently unknown. One possible explanation is that the metabolism and/or disposition of dizocilpine differs between male and female rats. It has been reported that female rats are more sensitive to the behavioral effects of PCP than male rats, as evidenced by hyperactivity, stereotyped behaviors, motor incoordination, tremor, salivation, and ataxia and that the concentrations of PCP in the female rat brain and plasma are higher than those in male rats (Nabeshima et al. 1984). This suggests that gender differences in the pharmacological actions of PCP could depend largely upon differences in the biotransformation (e.g., hepatic metabolizing system) of PCP. It has also been reported that females are more sensitive than males to dizocilpine-induced behavioral changes in rats (Honack and Loscher 1993; Andine et al. 1999) and that female and male rats that received the same dose of dizocilpine differed most dramatically with respect to the concentrations of dizocilpine in blood and brain (Andine et al. 1999). Similar to PCP, dizocilpine is also metabolized in the liver, and the pharmacokinetic differences between male and female rats may be explained by a low capacity for metabolism of dizocilpine by the liver in female rats, which would imply that the half life of dizocilpine in female rats is longer than that in male rats. Therefore, dizocilpine may be active in the female rat brain at a higher concentration for a longer period of time, and, consequently, may exert greater behavioral and biochemical effects at lower doses. Taken together, it is likely that the pharmacokinetic differences between male and female rats may contribute to the gender differences in the expression of BDNF mRNA and hsp-70 mRNA in the rat retrosplenial cortex induced by dizocilpine.

Another possible explanation is the difference in gonadal hormones in male and female rats. It has been demonstrated that adult female rats exhibit greater hormonal and behavioral responses to dizocilpine than male rats (Fleischmann et al. 1991; Blanchard et al. 1992; Honack and Loscher 1993). It has been also been reported that acute administration of PCP or dizocilpine increases plasma levels of such stress hormones as ACTH and corticosterone (Pechnick et al. 1989; Shirayama et al. 1999). Moreover, Honack and Loscher (1993) reported that gender differences in dizocilpine-induced behavioral changes in rats could occur within minutes of drug injection, which undermines the importance of any differences in the metabolism of dizocilpine. This suggests that gonadal hormones may play a more important role in gender differences in the expression of BDNF mRNA and hsp-70 mRNA in the rat retrosplenial cortex induced by dizocilpine. Further studies will be necessary to examine the role of gonadal hormones in modulating the response to dizocilpine.

Very recently, it has been reported that levels of BDNF, but not nerve growth factor and neurotrophin-3, are elevated specifically in the anterior cingulate cortex and hippocampus of post-mortem brain tissue of schizophrenic patients (Takahashi et al. 2000). These two regions are part of the Papez-like limbic circuit (entorhinal cortex, hippocampus, subiculum, mamillary body, anterior thalamic nucleus, anterior cingulate cortex, posterior cingulate/retrosplenial cortex), which plays a pivotal role in emotion and in learning and memory processes (Papez 1937; Sutherland and Hoesing 1993). These regions show altered function that is not only associated with the positive symptoms of schizophrenia, but also with the psychosis induced by ketamine, as shown in studies of schizophrenic patients and normal controls (Lahti et al. 1995). Furthermore, it is also known that the expression of BDNF can be influenced by various hormones, cytokines, and neurotransmitters, among which the strongest positive regulator might be the excitatory amino acid glutamate (Zafra et al. 1990).

Interestingly, it was recently reported that BDNF produced a threefold increase in glutamate-evoked, but not acetylcholine-evoked, current as well as increasing the open probability time of the NMDA receptor (Levine et al. 1998). Dizocilpine blocked the BDNF-induced enhancement in synaptic transmission, suggesting that BDNF may be an endogenous ligand that regulates NMDA receptor-dependent synaptic plasticity (Levine et al. 1998). In addition, a variety of anomalies in the anterior cingulate cortex of schizophrenics have been reported, which include a decrease in density of nonpyramidal neurons and increased $GABA_A$ receptor binding activity (review by Benes 2000). Based on the glutamate dysfunction hypothesis of schizophrenia, the abnormality in BDNF levels observed in the anterior cingulate cortex and hippocampus may be associated with glutamatergic dysfunction within corticolimbic structures of schizophrenics and might provide a molecular basis for both the structural and phenotypic impairments seen in the schizophrenia (Takahashi et al. 2000).

In summary, we found the age and gender differences in the expression of BDNF mRNA and hsp-70 mRNA in rat retrosplenial cortex following administration of dizocilpine (0.3, 1.0, or 3.0 mg/kg). Furthermore, the present study suggests that age and gender differences in the expression of BDNF mRNA and hsp-70 mRNA in rat retrosplenial cortex by administration of dizocilpine might be associated with these differences in the dizocilpine-induced neuronal injury in the same region.

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REFERENCES

Andine P, Widermark N, Axelsson R, Nyberg G, Olofsson U, Martensson E, Sandberg M (1999): Characterization of MK-801-induced behavior as a putative rat model of psychosis. J Pharmacol Exp Ther 290:1393–1408

Auer RN (1996): Effect of age and sex on N-methyl-D-aspartate antagonist-induced neuronal necrosis in rats. Stroke 27:743–746

Benes FM (2000): Emerging principles of altered neural circuitry in schizophrenia. Brain Res Rev 31:251–269

Blanchard DC, Blanchard RJ, Carobrez ADP, Veniegas R, Rodgers RJ, Shepherd JK (1992): MK-801 produces a reduction in anxiety-related antipredator defensiveness in male and female rats and a gender-dependent increase in locomotor behavior. Psychopharmacology 108:352–362

Baldridge EB, Bessen HA (1990): Phencyclidine. Emerg Med Clin North Am 8: 541–550

Brown IR (1994): Induction of heat shock genes in the mammalian brain by hyperthermia and tissue injury. In Mayer J, Brown IR (eds), Heat Shock Proteins in the Nervous System. London, UK, Academic Press, pp 31–53

Castren E, da Penha Berzaghi M, Londholm D, Thoenen H (1993): Differential effects of MK-801 on brain-derived neurotrophic factor mRNA levels in different regions of the rat brain. Exp Neurol 122:244–252

Coyle JT (1996): The glutamatergic dysfunction hypothesis for schizophrenia. Harvard Rev Psychiat 3:241–253

Ellison G (1995): The N-methyl-D-aspartate antagonists phencyclidine, ketamine, and dizocilpine as both behavioral and anatomical models of the dementias. Brain Res Rev 20:250–267

Ernfors P, Bengzon J, Kokaia Z, Persson H, Lindvall O (1991): Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. Neuron 17:165–176

Farber NB, Newcomer JW, Olney JW (1998): The glutamate synapse in neuropsychiatric disordrers—Focus on schizophrenia and Alzheimer's disease. Prog Brain Res 116:421–437

Farber NB, Wozniak DF, Price MT, Labruyere J, Huss J, Peter HS, Olney JW (1995): Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: Potential relevance to schizophrenia. Biol Psychiat 38:788–796

Fix AS, Wozniak DF, Truex LL, McEwen M, Miller JP, Olney JW (1995): Quantitative analysis of factors influencing neuronal necrosis induced by MK-801 in the rat posterior cingulate/retrosplenial cortex. Brain Res 696:194–204

Fleischmann A, Vincent PA, Etgen AM 1991: Effects of noncompetitive NMDA receptor antagonists on reproductive and motor behaviors in female rats. Brain Res 568:138–146

Hashimoto K, Minabe Y, Iyo M (1998a): Expression of brain-derived neurotrophic factor (BDNF) mRNA in rat retrosplenial cortex following administration of PCP. Addiction Biol 3:79–83

Hashimoto K, Tomitaka S, Narita N, Minabe Y, Iyo M, Fukui S (1996): Induction of heat shock protein (HSP)-70 in posterior cingulate and retrosplenial cortex of rat brain by dizocilpine and phencyclidine: Lack of protective effects of σ receptor ligands. Addiction Biol 1:61–70

Hashimoto K, Tomitaka S, Bi Y, Narita N, Minabe Y, Iyo M (1997): Rolipram, a selective phosphodiesterase type-IV inhibitor, prevents induction of heat shock protein HSP-70 and hsp-70 mRNA in rat retrosplenial cortex by dizocilpine. Eur J Neurosci 9:1891–1901

Hashimoto K, Watanabe K, Nishimura T, Iyo M, Shirayama Y, Minabe Y (1998b): Behavioral changes and expression of heat shock protein hsp-70 mRNA, brain-derived neurotrophic factor mRNA, and cyclooxygenase-2 mRNA in rat brain following seizures induced by systemic administration of kainic acid. Brain Res 804:212–223

Honack D, Loscher W (1993): Sex differences in NMDA receptor-mediated responses in rats. Brain Res 620:167–170

Hughes P, Dragunow M, Beiharz E, Lawlor P, Gluckman P (1993): MK-801 induces immediate-early gene proteins and BDNF mRNA in rat cerebrocortical neurons. NeuroReport 4:183–186

Isackson P, Huntsman MM, Murray KD, Gall CM (1991): BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: Temporal patterns of induction distinct from NGF. Neuron 6:937–948

Javitt DC, Zukin SR (1991): Recent advances in the phencyclidine model of schizophrenia. Am J Psychiat 148:1301–1308

Krystal JH, Karper L, Pseibyl JP, Freeman GK, Delaney R, Bremmer JD, Heninger GR, Bowers MB, Charney DS (1994): Subanesthetic effects of noncompetitive NMDA antagonist, ketamine, in humans. Arch Gen Psychiat 51:199–214

Lahti AC, Holcomb HH, Medoff DR, Tamminga CA (1995): Ketamine activates psychosis and alters limbic blood flow in schizophrenia. NeuroReport 6:869–872

Levine ES, Crozier RA, Black IB, Plummer MR (1998): Brain-derived neurotrophic factor modulates hippocampal synaptic transmission by increasing N-methyl-D-aspartic acid receptor activity. Proc Natl Acad Sci USA 95:10235–10239

Lindsay RM, Wiegand SJ, Atlar CA, DiStefano PS (1994): Neurotrophic factors: From molecule to man. Trends Neurosci 17:182–190

Lindvall O, Kokaia Z, Bengzon J, Elmer E, Kokaia M (1994): Neurotrophins and brain insults. Trends Neurosci 17:490–496

Luby ED, Cohen BD, Rosenbaum G, Gottilieb JS, Kelly R (1959): Study of a new schizophrenomimetic drug—sernyl. Arch Neurol Psych 81:363–369

Massa SM, Swanson RA, Sharp FR (1996): The stress gene response in brain. Cereb Brain Metab Rev 8:95–158

Nabeshima T, Yamaguchi K, Yamada K, Hiramatsu M, Kuwabara Y, Furukawa H, Kameyama T (1984): Sex-dependent differences in the pharmacological actions

and pharmacokinetics of phencyclidine. Eur J Pharmacol 97:217–227

Olney JW, Farber NB (1995): Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiat 52:998–1007

Olney JW, Labruyere J, Price MT (1989): Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 244:1360–1362

Papez JW (1937): A proposed mechanism of emotion. Arch Neurol Psychiat 38: 725–743

Paxinos G, Watson C (1997): The Rat Brain in Stereotaxic Coordinates. San Diego, CA, Academic Press.

Pechnick RN, George R, Poland RE (1989): Characterization of the effects of the acute and repeated administration of MK-801 on the release of adrenocorticotropin, corticosterone, and prolactin in the rat. Eur J Pharmacol 164:257–263

Reich DL, Silvay G (1989): Ketamine: An update on the first 20 years of clinical experience. Can J Anaesth 36:186–197

Sharp FR, Jasper P, Hall, Noble J, Sagar SM (1991): MK-801 and ketamine induce heat shock protein HSP70 in injured neurons in posterior cingulate and retrosplenial cortex. Ann Neurol 30:801–809

Sharp FR, Sagar SM (1994): Alterations in gene expression as an index of neuronal injury: Heat shock and the immediate early gene response. Neurotoxicology 15:51–60

Shimada A, Mukhin A, Ingram DK, London ED (1997): N-methyl-D-aspartate receptor binding in brains of rats at different ages. Neurobiol Aging 18:329–333

Shirayama Y, Hashimoto K, Shirayama M, Watanabe K, Ogawa T, Higuchi T, Minabe Y (1999): Effects of acute and chronic administration of methamphetamine or phencyclidine on plasma adrenocorticotropin, corticosterone, and progesterone in rat. Addiction Biol 4:345–350

Sutherland RJ, Hoesing JM (1993): Posterior cingulate cortex and spatial memory: A microlimnology analysis. In Vogt BA, Gabriel M (eds), Neurobiology of Cingulate Cortex and Limbic Thalamus. Boston, Birkhauser, pp 461–477

Takahashi M, Shirakawa O, Toyooka K, Kitamura N, Hashimoto T, Maeda K, Koizumi S, Wakabayashi K, Takahashi H, Someya T, Nawa H (2000): Abnormal expression of brain-derived neurotrophic factor and its receptor in the corticolimbic system of schizophrenic patients. Mol Psychiat 5:293–300

Tamminga CA (1998): Schizophrenia and glutamatergic transmission. Crit Rev Neurobiol 12:21–36

Welch MJ, Correa GA (1980): PCP intoxication in young children and infants. Clin Pedistr 19:510–514

White PF, Way WL, Trevor AJ (1982): Ketamine—Its pharmacology and therapeutic uses. Anesthesiology 56:119–136

Zafra F, Hengerer B, Leibrock J, Thoenen H, Lindholm D (1990): Activity- dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. EMBO J 9:3545–3550