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Long-lasting, distinct changes in central opioid receptor and urinary bladder functions in models of schizophrenia in rats

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ABSTRACT

Ketamine treatments and social isolation of rats reflect certain features of schizophrenia, among them altered pain sensitivity. To study the underlying mechanisms of these phenomena, rats were either housed individually or grouped for 33 days after weaning, and treated with either ketamine or saline for 14 days. After one month re-socialization, the urinary bladder capacity by ultrasound examination in the anesthetized animals, and changes of μ-opioid receptors by saturation binding experiments using a specific μ-opioid agonist [^{3}H]DAMGO were determined. G-protein signaling was investigated in DAMGO-stimulated [^{35}S]GTPγS functional assays. Ketamine treatment significantly decreased the bladder volume and isolation decreased the receptor density in cortical membranes. Among all groups, the only change in binding affinity was an increase induced by social isolation in the cortex. G-protein signaling was significantly decreased by either ketamine or social isolation in this tissue. Ketamine treatment, but not housing, significantly increased μ-opioid receptor densities in hippocampal membranes. Both ketamine and isolation increased the efficacy, while the potency of signaling was decreased by any treatment. Ketamine increased the receptor density and G-protein activation; while isolation decreased the efficacy of G-protein signaling in hippocampal membranes. The changes in the co-treated group were similar to those of the isolated animals in most tests. The distinct changes of opioid receptor functioning in different areas of the CNS may, at least partially, explain the augmented nociceptive threshold and morphine potency observed in these animals. Changes in the relative urinary bladder suggest a detrusor hyperreflexia, another sign of schizophrenia.

1. Introduction

A major challenge in modern medicine is to understand cellular and molecular mechanisms underlying common mental illnesses such as schizophrenia, which involve complicated genetic and environmental determinants (Maynard et al., 2001). Clinical reports suggest that many patients with schizophrenia are less sensitive to pain than other individuals, and this is associated with increased morbidity and mortality (Dworkin, 1994; Jochum et al., 2006). There is mounting evidence that the glutamate neurotransmitter system, in particular N-methyl-D-aspartic acid (NMDA) receptor hypofunction, might be a contributing factor leading to symptoms of schizophrenia (Stone et al., 2007). NMDA receptor expression and localization are disrupted in patients with schizophrenia (Kristiansen et al., 2007), and exposing rodents to NMDA receptor antagonists leads to certain schizophrenia-like behaviors (Becker and Grecksch, 2004; Wedzony et al., 2008; Guo et al., 2009).

Social isolation especially that of young animals causes behavioral changes, which include decreased pain sensitivity, increased spontaneous locomotor activity, and deficits in learning and memory (Gentsch et al., 1988; Paulus et al., 2000; Weiss and Feldon, 2001; Varty et al., 2006). Social isolation results in altered neurochemical systems too, including dopaminergic and serotoninergic functions (Jones et al., 1992; Crespi et al., 1992; Fone et al., 1996). These changes have been suggested to be similar to the changes seen in patients with schizophrenia (Varty et al., 1999; Paulus et al., 2000).

It has shown that sub-chronic treatment with the NMDA receptor antagonist, ketamine and subsequent social isolation produced changes in the pain sensitivity in adult rats (Becker et al., 2006). Recently, we have found that juvenile isolation for four weeks produced a long-lasting decrease mainly in the C-fiber-mediated pain sensitivity (Tuboly et al., 2009). It is well known that the selective disturbance of C-fibers by

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capsaicin treatment increases the urinary bladder capacity (Maggi et al., 1989), while detrusor hyperreflexia has been found in schizophrenic patients (Bonney et al., 1997). The effects of these treatments on the urinary bladder capacity were determined to characterize further our schizophrenic model.

It has been shown that both ketamine treatment and social isolation potentiated the antihyperalgesic effect of morphine (Tuboly et al., 2009). Alterations of the opioidergic systems fit well in the pathophysiology of schizophrenia (Davis et al., 1979; Wiegant et al., 1992; Bernstein et al., 2002; Becker et al., 2006; Tuboly et al., 2009; Becker et al., 2009). Becker et al. have shown significant effects of ketamine treatment and social isolation in the μ-opioid receptor density and functional activity in the central nervous system (CNS) of adult rats (Becker et al., 2006). Since juvenile social isolation and/or ketamine treatment have changed the pain sensitivity and the potency of morphine (Tuboly et al., 2009), we have chosen this combination as a model system of schizophrenia. The second goal was to investigate changes in the binding and signaling properties of the μ-opioid receptors in different parts of the CNS, which are involved in the pain transmission, i.e. cerebral cortex, hippocampus and spinal cord.

2. Materials and methods

2.1. Animals

After institutional ethical approval had been obtained from the animal care committee, male Wistar rats were used. After weaning (on day 21 of age: 1st day) rats were either housed individually or grouped for 33 days (1–33) in cages measuring 40×13×18 cm and 40×28×19 cm, respectively (l×w×h). The animals were treated daily from day 7 to day 20 with either ketamine (30 mg/kg intraperitoneally, i.p.) or saline. In total, the rats received 14 injections. The duration of the treatment was adapted from our recent study (Tuboly et al., 2009). Four experimental groups were studied: saline + non-isolated (sal-niso), ketamine + non-isolated (ket-niso), saline + isolated (sal-iso), and ketamine + isolated (ket-iso). The groups were matched according to body weight (55 ± 0.6 g). Rats were kept in a temperature controlled room (22 ± 1 °C); food and water were available ad libitum. The cages were located together in racks so that auditory and olfactory contact was maintained. The total number of animals as well as their suffering was minimized whenever possible. At the age of 8 weeks the animals were resocialized and they had at least a 6 week resting period.

2.2. Chemicals

The drugs employed were ketamine hydrochloride (Calypsol, Richter Gedeon RT, Budapest, Hungary) and dexmedetomidine hydrochloride (as a generous gift from Orion-Pharmos Pharmaceuticals Turku, Finland). Physiological saline served as a control. Freshly prepared solutions were administered i.p. or subcutaneously (s.c.) injected at a volume of 4 or 2 ml/100 g body weight, respectively. [^{3}H]Tyr-Gly-(NMe)PHe-Gly-ol,[^{3}H]DAMGO (41 Ci/mmol) was synthesized in the Isotope Laboratory of the Biological Research Center (Szeged, Hungary). Guanosine-5′-O-(3-[^{35}S]thio)triphosphate,[^{35}S]GTPγS (1204 Ci/mmol) was purchased from the Isotope Institute Ltd. (Budapest, Hungary). Bovine serum albumin (BSA-essentially fatty acid free), ethylene-bis (oxyethylenenitrilo) tetraacetic acid (EGTA), guanosine 5′-diphosphate sodium salt (GDP), guanosine 5′-[γ-thio]triphosphate tetralithium salt (GTP-γ-S-Li$_4$), magnesium chloride hexahydrate ($MgCl_2 \times 6\ H_2O$), sodium chloride (NaCl) and Tris(hydroxymethyl) aminomethane (Tris, free base) were purchased from Sigma-Aldrich (Budapest, Hungary). Bradford reagent was from Bio-Rad Laboratories (Hercules, CA, USA). Tyr-Gly-(NMe)PHe-Gly-ol,DAMGO was purchased from Bachem AG (Bubendorf, Switzerland).

2.3. Ultrasound examination

Earlier studies proved the reliability of this method for the calculation of urinary bladder volume (Horvath et al., 1994; Keirstead et al., 2005). The experiments were performed at the age of week 14 of rats (n = 8–12/group) as we published (Horvath et al., 1994). The animals were anesthetized with dexmedetomidine (150 μg/kg s.c.), and the abdominal region of each rat was shaved. We used sonography – 7.5 MHz linear passed array transducer (Hitachi EUB 405) – to image the urinary bladder of the rats. Each bladder volume was estimated from a longitudinal and a transverse image section. The transducer was moved from side to side to find the largest cross-sectional area by application of a slight pressure. Two photographs were required to determine the length and the transverse diameter of the urinary bladder. Bladder volumes were assessed when the urine appeared (volume threshold), and two more times (about 30–30 min later) again in each animal, and the mean of these values was analyzed. The bladder volume was estimated by substituting the diameters into the ellipsoid equation formula:

$$V = a \times b \times c \times \pi / 6.$$

The variables are the length of the major axes. Since the bladder volume increases significantly with body weight (Horvath et al., 1994), we calculated the volume (ml) for 100 g body weight.

Relative bladder volume:

$$V_{100\,g} = (bladder\,volume * 100) / (body\,weight).$$

2.4. Membrane preparation

2.4.1. Cerebral cortex and hippocampus

At the age of week 15 animals were sacrificed, their cerebral cortex and hippocampus were dissected (n = 4/group) and crude membrane fractions were prepared as described earlier (Bozu et al., 1997). Briefly, the brain areas were homogenized in 30 volumes (v/w) of ice-cold 50 mM Tris–HCl pH 7.4 buffer with a teflon-glass Braun homogenizer operating at 1500 rpm. The homogenate was centrifuged at 20,000×*g* for 25 min at 4 °C, the resulting supernatant carefully discarded and the pellet taken up in the original volume of Tris–HCl buffer. After homogenization with a Dounce, the homogenate was incubated at 37 °C for 30 min in a shaking water-bath. Centrifugation was then repeated as described above. The final pellet was suspended in 5 volumes of 50 mM Tris–HCl pH 7.4 buffer containing 0.32 M sucrose, frozen in liquid N_2 and stored at −80 °C. Prior to the experiments, an appropriate aliquot was melted, diluted with 5-fold Tris–HCl buffer and centrifuged at 20,000×*g* for 25 min to remove sucrose. The protein content of the membrane preparation was determined by the method of Bradford, BSA being used as a standard (Bradford, 1976).

2.4.2. Spinal cord

Rat spinal cords (n = 4/group) were dissected and stored at −80 °C for several weeks. They were thawed before use and homogenized in 10 volumes (v/w) of ice-cold 50 mM Tris–HCl (pH 7.4) with 5 strokes in a teflon-glass Braun homogenizer operating at 700 rpm. The homogenate was centrifuged at 5000×*g* for 10 min at 4 °C. The supernatant was carefully decanted and stored on ice, and the pellet was taken up in the original volume of Tris buffer (50 mM, pH 7.4) and centrifuged again as above. The combined supernatants of the two centrifugation steps were centrifuged at 20,000×*g* for 25 min at 4 °C. The resulting pellet was taken up in the original volume of 50 mM Tris–HCl (pH 7.4) and incubated for 30 min at 37 °C in a shaking water bath. Centrifugation was then repeated as described above. The final pellet was taken up in 30 volumes (v/w) of ice-cold

50 mM Tris–HCl (pH 7.4) and immediately used in binding assays. The protein content of the membrane preparation was determined by method of Bradford, BSA being used as a standard (Bradford, 1976).

2.5. In vitro experimental section

2.5.1. Saturation binding measurement

Saturation binding experiments were performed with increasing concentrations (0.25–10 nM) of [^{3}H]DAMGO and appropriated membrane preparations (≈150 μg proteins) in 50 mM Tris–HCl (pH 7.4) buffer in a final volume of 1 ml. Nonspecific binding was determined with 1 μM unlabeled DAMGO and subtracted from the total value to give the specific binding. Incubation was performed at 25 °C for 60 min. The reaction was stopped by diluting the samples with 5 ml of ice-cold 50 mM Tris–HCl (pH 7.4) buffer, followed by rapid filtration through Whatman GF/C glass fiber filters (Whatman LTD, Maidstone, England) with a Brandel M24-R Cell Harvester (Gaithersburg, MD, USA). Filters were washed twice with 5 ml of ice-cold 50 mM Tris–HCl (pH 7.4) buffer, air-dried and counted in a toluene-based scintillation cocktail in a Wallac 1409 Counter (Wallac, Turku, Finland). All assays were performed in duplicate and repeated at least three times. Only specific binding is reported. Results are the mean ± S.E.M. of at least three independent experiments all performed in duplicate.

2.5.2. Ligand-stimulated [^{35}S]GTPγS functional assay

Membranes (≈10 μg of proteins) were incubated with 0.05 nM [^{35}S]GTPγS and increasing concentrations (10^{-10}–10^{-5} M) of unlabeled DAMGO in the presence of 30 μM GDP, 100 mM NaCl, 5 mM $MgCl_2$ and 1 mM EGTA in a total volume of 1 ml in 50 mM Tris–HCl (pH 7.4) buffer as published (Bozo et al., 1994; Fabian et al., 2002). Nonspecific binding was determined with 10 μM GTPγS and subtracted. Bound and free [^{35}S]GTPγS were separated by vacuum filtration through Whatman GF/F filters with Brandel M 24-R Cell Harvester as above. Basal activities, assessed in the absence of opioids, were defined as 0%. Results are expressed as % stimulation of basal and are the means ± S.E.M. of at least three independent experiments, all performed in triplicate.

2.6. Data analysis

Data analyses were performed with the GraphPad Prism 4.0 software (GraphPad Software Inc. La Jolla, CA, US). Non-linear regression analysis of the direct saturation isotherms was performed to obtain the equilibrium dissociation constant (K_D,) and receptor density (B_{max}) values. E_{max} (efficacy, maximal stimulation) and $-\log EC_{50}$ (the negative logarithm of concentration of the agonist required to achieve 50% of the maximal stimulation, i.e. potency) values were determined by nonlinear regression of the dose–response curves in DAMGO-stimulated [^{35}S]GTPγS functional assays. Statistically significant effects of the treatments (saline or ketamine) and conditions (non-isolated or isolated), and the possible interactions between them were calculated using two-way ANOVA followed by Bonferroni post-hoc tests.

3. Results

3.1. Urinary bladder volume

There were no significant differences in the body weight between the different groups (mean: 449 ± 4.9 g; $p = 0.39$) (Fig. 1A). As was observed in our earlier study, dexmedetomidine caused a long-lasting hypnotic–anesthetic effect and it also produced diuresis and passive incontinence which allowed the ultrasound examination of the urinary bladder (Horvath et al., 1994). The urine appeared about 30 min after the administration of dexmedetomidine. Although dribbling of urine was observed almost continuously, there were no significant differences between the bladder volumes at the three time points, that is, the bladders were overfilled at each recording time (data are not shown). Two-way ANOVA revealed a significant effect of housing condition × treatment interaction ($F_{1,34} = 4.49$; $P<0.05$). Post hoc comparison showed that the bladder was significantly smaller in the ket-niso group compared to the control animals, and there was a tendency for a decreased urinary bladder volume in the other treated groups as well (Fig. 1B).

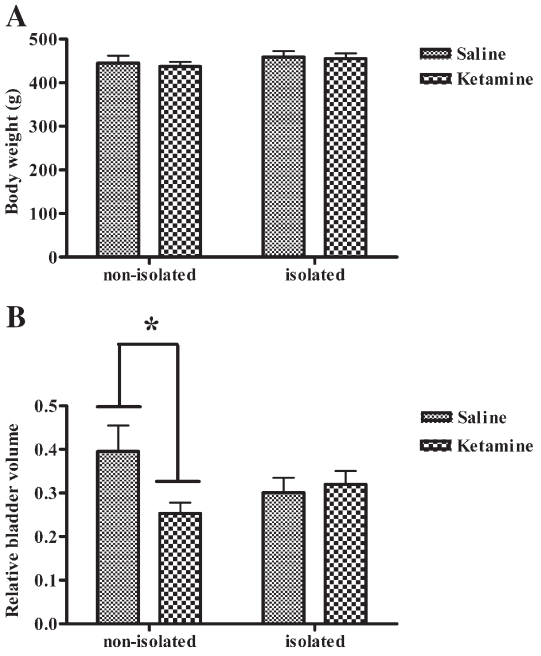

Fig. 1. The body weight at the age of week 14 (A) and the relative bladder volumes (B) registered 3 times during anesthesia after dribbling started. Results are expressed as mean for 8–12 rats. Significance is set at *$P<0.05$.

3.2. In vitro results (opioid binding results)

3.2.1. Changes in the binding parameters of μ-opioid receptors

Changes due to the treatments and conditions were tested by [^{3}H]DAMGO saturation binding assays in various CNS regions. DAMGO is a specific μ-opioid agonist binding with high affinity to these receptors. As regards the B_{max} in the cerebral cortex, two-way ANOVA revealed a significant effect of housing condition ($F_{1,8} = 89.16$; $P<0.0001$; Fig. 2A). Post-hoc analysis showed that both sal-iso and ket-iso groups had much lower binding capacity than their appropriated counterparts. Similarly, a significant effect of the housing condition was observed on the binding affinity ($F_{1,8} = 6.37$; $P<0.05$). Post-hoc comparison revealed that social isolation decreased the K_D in the sal-iso group, but this effect of social isolation was blunted by ketamine (Table 1). Therefore, social isolation decreased the number of binding sites and increased the binding affinity in the cerebral cortex.

ANOVA showed significant effects of condition ($F_{1,8} = 14.38$; $P<0.01$) and interaction ($F_{1,8} = 11.32$; $P<0.01$) for changes in the binding parameters of μ-opioid receptors in the hippocampal membranes. Post-hoc analysis showed that ketamine treatment significantly ($F_{1,8} = 6.85$; $P<0.05$) enhanced the B_{max} by itself, and this effect of ketamine was inhibited by social isolation (Fig. 2B). Neither social isolation nor ketamine treatment had significant effects on the binding affinity (Table 1).

Ketamine treatment ($F_{1,8} = 24.64$; $P<0.01$) but not social isolation increased the binding capacity in the spinal cord. Post-hoc analysis revealed that ketamine was effective only in the grouped animals, and this effect was inhibited by social isolation (Fig. 2C). The binding affinity was not significantly changed by any treatment, although

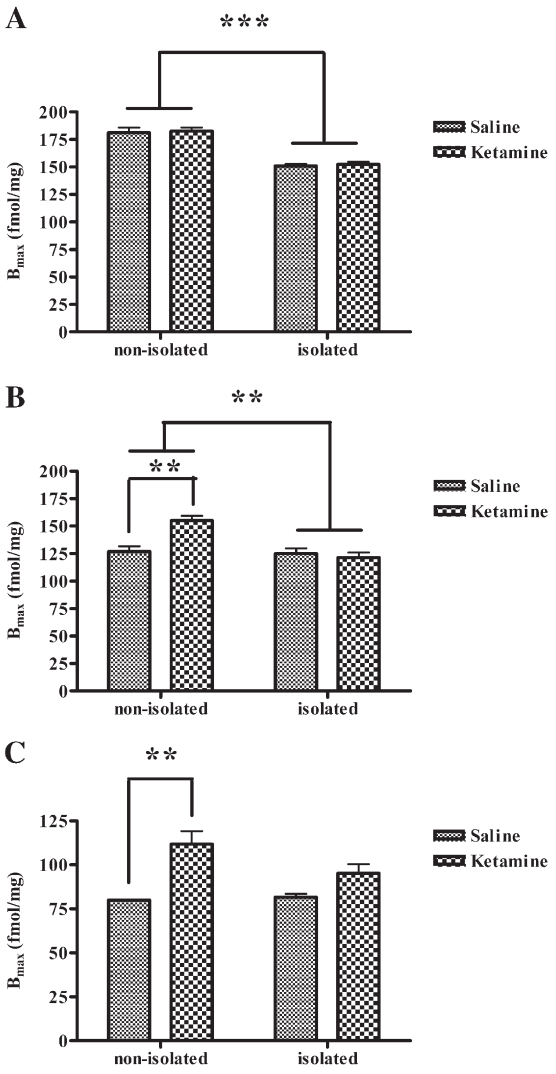

Fig. 2. Changes in the receptor density (B_{max}) due to ketamine treatment in non-isolated and isolated rats. Saturation binding curves with varying concentrations (0.25–10 nM) of [^{3}H]DAMGO were assessed in the crude membranes of cerebral cortex A), hippocampus B) and spinal cord C). The nonspecific binding was measured with 1 μM unlabeled DAMGO and subtracted. Means ± S.E.M., n = 3, performed in duplicate. Significance is set at **P<0.01, and ***P<0.001.

there was a tendency for a decrease in the ket-iso group (Table 1). Therefore, only ketamine treatment but not housing, changed (i.e. increased) the binding capacity of μ-opioid receptors in hippocampal and spinal cord membranes.

3.2.2. Changes in G-protein signaling of μ-opioid receptors

We utilized the ligand-stimulated [^{35}S]GTPγS functional assay to examine the effect of ketamine and/or isolation on μ-opioid receptor signaling. This assay relies on agonist-promoted GDP/GTP exchange occurring at the G-protein level within the receptor/G-protein complex. DAMGO stimulated [^{35}S]GTPγS binding with an EC_{50} of 342–429 nM above basal activities in the studied tissues. Analyzing the G-protein activation in the cortex showed significant effects of housing ($F_{1,8} = 11.20$; $P<0.05$) and interaction ($F_{1,8} = 23.81$; $P<0.01$). Post-hoc analysis showed that both ketamine and isolation produced decreased stimulations (E_{max}) compared to the sal-niso group, but the combination of these two effects did not cause further changes (Fig. 3A). Neither treatment nor isolation had any effect on the potency ($-\log EC_{50}$) of DAMGO (Table 2). Thus, the degree of G-protein activation decreased, but the potency of the G-protein activation did not change due to ketamine treatment and/or isolation in the cerebral cortex.

Table 1
The equilibrium dissociation constant, K_D values of [^{3}H]DAMGO binding in various tissues.

	K_D (nM)			
	Non-isolated + saline	Non-isolated + ketamine	Isolated + saline	Isolated + ketamine
Cortex	0.84 ± 0.08	0.71 ± 005	0.58 ± 0.03[a]	0.69 ± 0.05
Hippocampus	0.69 ± 0.11	1.01 ± 0.10	0.87 ± 0.13	0.87 ± 0.13
Spinal cord	1.94 ± 0.21	1.94 ± 0.42	1.92 ± 0.16	2.76 ± 0.44

K_D values were calculated by GraphPad Prism computer program from the same [^{3}H] DAMGO direct saturation-plots as for obtaining B_{max} values, the latter plotted in Fig. 2. Data are means ± S.E.M. of at least 3 independent experiments each performed in duplicate. Statistical analysis was performed as described in Materials and methods, [a]: $P<0.05$ vs. non-isolated + saline.

Significant effect of housing condition ($F_{1,12} = 22.13$; $P<0.001$) and housing condition × treatment interaction ($F_{1,12} = 46.34$; $P<0.001$) was

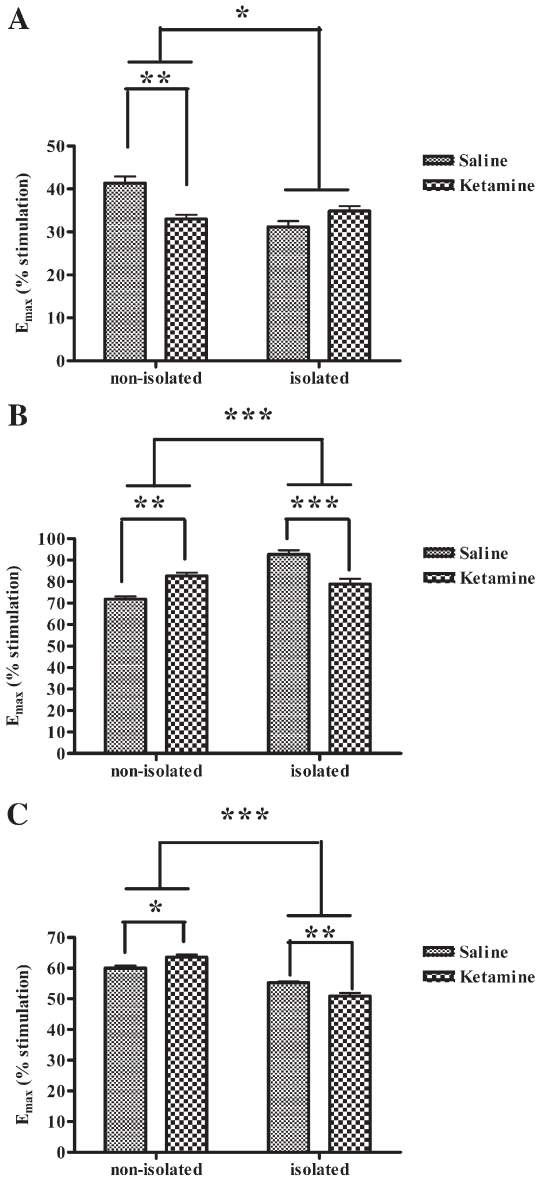

Fig. 3. DAMGO-stimulated [^{35}S]GTPγS binding in crude membranes of cerebral cortex A), hippocampus B) and spinal cord C) of non-isolated and isolated rats. Nonspecific binding was determined with 10 μM GTPγS and subtracted. Means ± S.E.M., n ≥ 3, performed in triplicate. Significance is set at *P<0.05, **P<0.01, and ***P<0.001.

Table 2
Potency, EC_{50} values of DAMGO-stimulated [^{35}S]GTPγS binding assays in various tissues.

	$-\log EC_{50}$			
	Non-isolated + saline	Non-isolated + ketamine	Isolated + saline	Isolated + ketamine
Cortex	6.43 ± 0.11	6.65 ± 0.09	6.58 ± 0.12	6.70 ± 0.10
Hippocampus	6.60 ± 0.03	6.40 ± 0.04[a]	6.29 ± 0.04[c]	6.28 ± 0.06[d]
Spinal cord	6.35 ± 0.04	6.54 ± 0.03[b]	6.43 ± 0.02	6.40 ± 0.03[e]

Values of [$-\log EC_{50}$] together with E_{max} (see Fig. 3) values were obtained from the DAMGO-stimulated [^{35}S]GTPγS binding assays using GraphPad Prism computer program as described in Materials and methods and figure legend. Data are means ± S.E.M. of at least 3 independent experiments each performed in triplicate. Statistical analysis was performed as described in Materials and methods and set at [a]: $P<0.05$, [b]: $P<0.01$, [c]: $P<0.001$ vs. non-isolated + saline and [d]: $P<0.05$, [e]: $P<0.01$ vs. non-isolated + ketamine.

detected in the E_{max} values in the hippocampus. The post-hoc analysis showed that both ketamine treatment and isolation increased the maximal stimulation of G-proteins, and the co-treatment blunted the effects of social isolation (Fig. 3B). The $-\log EC_{50}$ value was decreased both by social isolation ($F_{1,12}=30.35$; $P<0.001$) and ketamine treatments ($F_{1,12}=9.70$; $P<0.01$; Table 2). Therefore, increased efficacy of G-protein activation with decreased sensitivity was observed after any treatment.

As regards the spinal cord, significant effect of housing condition ($F_{1,20}=114.67$; $P<0.001$) and housing condition × treatment interaction ($F_{1,20}=23.91$; $P<0.001$) was detected in the E_{max} values. Post-hoc analysis revealed that social isolation significantly decreased, while ketamine treatment increased the E_{max}, and the combination of the two effects produced the lowest value (Fig. 3C). Similarly, significant effect of housing condition ($F_{1,20}=6.17$; $P<0.05$) and housing condition × treatment interaction ($F_{1,20}=12.19$; $P<0.01$) was observed on the detected potency values, too. The post-hoc comparison revealed that the ketamine treatment by itself increased the activation of G-protein activation by DAMGO and isolation decreased this effect of ketamine (Fig. 3C). Therefore, an opposite effect was observed in ketamine vs. social isolation in the spinal cord.

4. Discussion

Functional and structural abnormalities of the cerebral cortex and the hippocampus in patients with schizophrenia have been reported and these structures have also been implied in the central control of nociception (Csernansky and Bardgett, 1998; Jentsch and Roth, 1999; Brooks and Tracey, 2005). The present study showed long-lasting and diverse changes in opioid receptor numbers and functions in the brain and the spinal cord in animals treated with ketamine and/or stressed by social isolation.

Schizophrenia is characterized by complex disturbances in multiple neurotransmitter systems, including the dopaminergic, serotoninergic, glutamatergic, and GABAergic neurotransmission in the brain (Yang et al., 1999; Jentsch and Roth, 1999; Gaspar et al., 2009). The investigation of the involvement of the opioid system in the schizophrenia models is of special interest because the opioid system may participate in the pathogenesis of decreased pain sensitivity in schizophrenic patients. While some investigators have found different concentrations and alterations in the number of binding sites and genetic polymorphisms, others have reported similar levels in schizophrenics and respective control populations (Gulya, 1990; Danos et al., 2002; Zhang et al., 2004). Elevated levels of beta-endorphin and instability of its secretion have been detected in schizophrenic patients (Wolkowitz et al., 1986; Mauri et al., 1998), while others found that the number of beta-endorphin-containing arcuate neurons was reduced in schizophrenic individuals (Bernstein et al., 2002). A recent human study has suggested that morphine can produce an antipsychotic effect (Quednow et al., 2008). As regards some animal models of schizophrenia, the endogenous μ-opioid receptor agonist endomorphin-1 did not alter PPI, but attenuated PPI deficits (Ukai and Okuda, 2003). Furthermore, the opioid receptor antagonist naloxone did not influence PPI in rats but prevented the loss of PPI induced by amphetamine (Swerdlow et al., 1991; Rosa et al., 2005).

NMDA receptor antagonists such as phencyclidine, ketamine and MK-801 induce behavioral syndrome in rodents may be a suitable model of schizophrenia, because they can mimic some of the positive and negative symptoms of schizophrenia in humans (Jentsch and Roth, 1999; Moghaddam and Jackson, 2003; Zuo et al., 2009). It has been hypothesized that NMDA antagonists produce neuronal injury by disinhibition GABA neurons leading to the release of acetylcholine and glutamate, the latter interacting with non-NMDA receptors (Olney and Farber, 1995). Furthermore, administration of NMDA receptor antagonists in the postnatal period accelerates neurodevelopmental apoptosis, leading to neuronal death (Gould et al., 1994; Wang et al., 2004). We found that μ-opioid receptor density increased after ketamine by itself treatment in the hippocampus and spinal cord, but not in the cerebral cortex. The decreased potency of DAMGO-stimulated [^{35}S]GTPγS binding suggested reduced coupling of μ-opioid receptors to G proteins after sub-chronic ketamine treatment in the hippocampus, while opposite changes were observed in the spinal cord. Becker et al. have found that the number of opioid receptors and their activation have not been influenced by ketamine treatment in the frontal cortex, while the binding sites decreased in the hippocampus and the relative efficacy of μ-opioid receptors was not modified (Becker et al., 2006). Several factors might be responsible for the different results, since the strain (Wistar vs. Sprague–Dawley), the age (young vs. adult) of the animals and the tissue samples (cortex vs. cerebral cortex) differed in these two studies. Our data suggest that ketamine treatment by itself induces long-lasting changes in the opioid receptor function both spinally and supraspinally, and these alterations might have led to the observed increased potency of morphine in vivo (Tuboly et al., 2009).

Rats normally live in social groups and prepubertal and pubertal intervals are critical for establishing social organization in this circumstance. Exposure of mammals to early-life social isolation adversely affects brain development and adult behavior, including glutamatergic hypofunction, reduction in prefrontal cortex volume and hippocampal synaptic plasticity and hyperfunction of mesolimbic dopaminergic systems (Geyer et al., 1993; Vanderschuren et al., 1997; Blanchard et al., 2001; Lapiz et al., 2003; Fone and Porkess, 2008; Tuboly and Horvath, 2009). It is inappropriate to describe isolation-reared rats as an ideal model for schizophrenia, nonetheless, isolation rearing of rats from weaning produces long-term changes in behavior including impaired PPI, social withdrawal and cognitive inflexibility which cover the three domains affected in schizophrenia (Fone and Porkess, 2008). Several studies have suggested the importance of endogenous opioid systems for the mediation of social interaction in young age (Adler et al., 1975; Kostowski et al., 1977; Panksepp, 1980; Winslow and Insel, 1991). Social isolation decreased the number but increased the affinity of opioid-receptor binding in the cerebral cortex, while the saturation experiments did not show any change in the hippocampus and the spinal cord. However, the efficacy of G-protein activation changed in an opposite manner in the latter two areas, i.e. the hippocampus showed an increased, while the spinal cord a decreased activation of G-proteins. As regards the $-\log EC_{50}$ values, only the hippocampus showed a decreased sensitivity. Earlier autoradiography studies showed that juvenile isolation caused region-specific changes in the number of μ-opioid binding sites in several brain areas (Van den Berg et al., 1999a, 1999b). Becker et al. have found that the number of opioid receptor binding sites has not changed in the frontal cortex and the hippocampus, while the relative efficacy of μ-opioid receptor activation increased in the frontal cortex, but did not modify in the hippocampus (Becker et al., 2006). The

different results might be explained by the differences in the age and strain of the animals as was discussed above. Furthermore, Becker et al. have used shorter isolation of adult rats (2 vs. 4.5 weeks), while in the present experiment, juvenile Wistar rats were isolated from the age of week 3, a period with high levels of social play (Vanderschuren et al., 1997; Pellis et al., 1997; Weiss and Feldon, 2001).

As regards the effects of ket-iso co-treatment on the opioid receptor function in the cortex, the number of the binding sites decreased (similarly to the isolation by itself), while the potency of G-protein signaling did not change (similarly to the other groups). In the hippocampus, the number and the affinity of the binding sites did not change due to ket-iso co-treatment (similarly to the isolation). The efficacy of G-protein activation by co-treatment was blunted compared to that by isolation itself. At spinal level, the density and the affinity of μ-opioid receptors in the keto-iso group did not change likewise in the isolated animals. Surprisingly, the G-protein activation was lowest in this group, while the sensitivity was similar to the sal-niso and sal-iso groups. Therefore, the changes in the ket-iso group were similar to the isolated animals in most tests, supporting that mainly isolation was responsible for the acute pain sensitivity changes. Since the potency of morphine increased in all the three pretreated groups (Tuboly et al., 2009), the diverse changes in these areas might be at least partially responsible for the observed effects. Only a few studies suggest interaction between NMDA treatment and social isolation. Indeed, social isolation and ketamine treatment in rats have been shown to induce emergence of behavioral abnormalities, such as hyperresponsiveness to stress, novelty, dopamine agonists or glutamate antagonist and enhancement of the isolation-induced locomotor hyperactivity (Lapiz et al., 2003; Becker et al., 2006, 2009). The number of binding sites increased in the frontal cortex, while decreased in the hippocampus, and the relative efficacy of μ-opioid receptors did not change (Becker et al., 2006). The differences might be explained by the differences in the age, strain of the animals and the isolation period as was discussed above. In addition, Becker et al. have used social isolation after ketamine or saline treatment.

As regards the bladder capacity in the different groups, the relative urinary bladder volume was decreased significantly by ketamine treatment. Urodynamic study suggested that incontinence represents detrusor hyperreflexia in a significant subset of schizophrenic patients (Bonney et al., 1997). Our recent study suggested that the responses to C-fiber related stimuli were impaired in our schizophrenia model (Tuboly et al., 2009). It is well-known that the impairment of C-fibers by capsaicin desensitization leads to increased urinary bladder volume (Maggi et al., 1989), therefore, we supposed that the disturbed C-fiber mediated response could lead to an increased urinary bladder capacity. We described a simple, noninvasive, reliable ultrasonographic method for the determination of urinary bladder volume in intact, anesthetized rats (Horvath et al., 1994). The urinary bladder capacity was not increased by these treatments; therefore, it is supposed that the disturbance in the C-fiber-related functions could not lead to an increased bladder volume (Tuboly et al., 2009). Even significant decrease in the bladder capacity was observed in the ketamine-treated animals suggesting detrusor hyperreflexia. Autonomic dysregulation in patients with schizophrenia is a well-known phenomenon, and this can produce gastric and urinary bladder dysmotility as well (Scigliano et al., 2008; Bonney et al., 1997; Peupelmann et al., 2009). It is suggested that the bladder overactivity observed in schizophrenic patients might be due to impaired cortical inhibition of the urinary bladder, and this condition could be treated with clozapine (Bonney et al., 1997; Kumar et al., 2007). As regards the effects of chronic NMDA-receptor antagonist treatment, earlier studies showed that administration of MK-801 for a week did not influence the bladder capacity in conscious adult Wistar rats, while ketamine causes bladder dysfunction in ketamine-dependent patients (Tanaka et al., 2003; Liao et al., 2011). Since anesthesia completely abolished the micturition reflex, the volume of the bladder can not provide direct information about the bladder overactivity. We suggest that the decreased cortical inhibition from weaning on, and for a long period might have led to changes in the capacity of urinary bladder. However, it is difficult to discuss the pathophysiology of urinary dysfunction in schizophrenia based only on the results of bladder capacity obtained in our study, therefore, further urodynamic studies – involving the investigation of urinary frequency/volume, bladder activity, and pressure changes – are required to clarify this phenomenon.

5. Conclusion

In conclusion, these results show that juvenile isolation and/or subchronic ketamine treatment produced significant long-lasting alteration in the opioid receptor functions both in the brain and in the spinal cord, suggesting that the decreased nociceptive responses observed in these animals were due, at least to a certain extent, to the modified opioid activation. The results underline the mediating role of opioid systems in adult behavior as a consequence of juvenile isolation. The changes in opioid receptor binding may be the result of alterations in treatment-induced peptides release, i.e. decreased activation of opiate peptide receptors by endogenous opioids released during these treatments could lead to receptor changes. Besides this effect on the opiate receptors, changes in nociception observed in these animals might also be due to modification in other neurotransmitter or hormonal systems able to act on the nociceptive pathways. Changes in the spinal cord might be especially important, since this is the site of the first synaptic connection of the pain stimuli from the body, and to our knowledge, there are no data about the μ-opioid receptor alterations in this respect. The exact mechanisms involved in the development of this effect are not completely known, and further investigations of this phenomenon are obviously required. Taken together, a shift in μ-opioid receptor density and efficacy might be one of the explanations for altered pain perception in our models. Although both ketamine treatment and isolation disturbed the function of μ-opioid receptors, no super-additive interaction was found indicating that isolation did not induce hypersensitivity to NMDA receptor antagonist treatment. We found that a slight disturbance in the peripheral C-fiber function did not lead to increased bladder volume, but similarly to schizophrenic patients sign of detrusor hyperreflexia was observed.

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