Expression of mRNA of Neurotrophic Factors and their Receptors are Significantly Altered After Subchronic Ketamine Treatment

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Abstract: The neurotrophic factors play an important role in the maintenance of neurone viability and neuronal communication which are considered to be altered in schizophrenia. Subchronic application of ketamine (Ket) was found to be a useful model in schizophrenia research. To further validate this model the mRNA levels of neurotrophic factors NGF, NT-3, and BDNF and their receptors TrkA, TrkB, and TrkC, respectively, were measured in different brain areas in Ket-pretreated rats subchronically dosed with the atypical antipsychotic drug risperidone (Ris).

With the exception of NGF in the frontal cortex, Ket pretreatment did change NGF, NT-3, and BDNF mRNA levels in the frontal cortex, the hippocampus, the striatum, the thalamus/hypothalamus region, and in the cerebellum. These changes correspond with changes at their tyrosine kinase receptors. Ris treatment normalised altered NT-3 levels in the hippocampus and balanced BDNF levels in the same structure. It was concluded that the Ket model might reflect distinct alterations in neurotrophic factor activity as found in schizophrenic patients and, moreover, that Ris treatment rebalances disturbed neurotrophic factor activity.

Key Words: Schizophrenia, ketamine, risperidone, neurotrophic factors, rat.

INTRODUCTION

Clinical and experimental research supported by several converging lines of evidence demonstrate a developmental aetiology of schizophrenia. According to the neurodevelopmental hypothesis, schizophrenia may be caused by subtle defects occurring in the course of brain development which act on their own or in conjunction with genetic factors resulting in a late-onset disorder that reflects abnormalities in neuronal functions [1-4].

Neurotrophins of the nerve growth factor family such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) which bind high-affinity tyrosine kinase receptors (TrkA, TrkB, TrkC) play a central role in neuronal differentiation and in the development of the central nervous system. Moreover, these factors are of special importance in the process of neuronal regeneration [5]. Thus, it was hypothesised that imbalances in the neurotrophic system might contribute to alterations in the brain which are closely connected with schizophrenia [5-12]. Post mortem studies have shown that BDNF mRNA and neurotrophins are changed in the hippocampus (Hip), the prefrontal cortex, and in the anterior cingulate cortex of schizophrenics [13, 14]. It was concluded that a dysfunction of BDNF and TrkB in the schizophrenic brain may be one of the factors involved in the pathogenesis of schizophrenia [12]. NT-3 concentrations of frontal and parietal cortical areas were significantly lower in patients than in controls [10]. In the frontal cortex (FC) of schizophrenics there was a 5.8-fold reduction in TrkC mRNA levels [15]. Plasma levels of NGF were found to be decreased in first-medicated schizophrenics [11]. Human studies on BDNF levels in schizophrenic patients showed different results. BDNF mRNA was found to be reduced in the Hippocampus and prefrontal cortex of patients with schizophrenia [16], whereas other studies reported elevated levels of BDNF protein in the anterior cingulate cortex and hippocampus of schizophrenic patients [17, 18]. In the peripheral blood of schizophrenic patients there were no significant differences in serum BDNF levels among non-medicated and medicated patients and normal controls [19]. Other studies found decreased BDNF in serum of patients treated with both atypical and typical antipsychotics or in first-episode schizophrenia patients [20, 21]. Although the emerging picture on the role of BNF in schizophrenia appears to be multi-faceted, all the data suggest that disturbances in the neurotrophin system may play a role in the complex nature of schizophrenia by altering the neuronal circuitry and/or parameters of neurotransmission that deserves further examination.

Different animal models have been developed for the study of schizophrenia. All these models reflect in part structural and functional aspects of the disease including dynamic changes in the neurotrophin system. A reduced basal level of BDNF mRNA was found in rats with neonatal ibotenic acid lesions of the ventral hippocampus [22], the dentate gyrus, and the prefrontal cortex [23]. Interestingly, the expression of BDNF was not altered by ventral hippocampal lesion in prefrontal and frontal cortex at pre- and postpubertal age [24]. In the medial prefrontal cortex and cingulate cortex the basal level of NGF inducible-factor B was significantly reduced in lesioned rats at post-pubertal age [25, 26]. Subchronic treatment with phencyclidine, which is a potent non-competitive antagonist of NMDA receptors presenting an-

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other validated animal model in schizophrenia research, was associated with increased expression of GDNF and its receptor mRNA in the substantia nigra compacta and ventral tegmental area [27]. This result was interpreted in terms of a modulating function of the GDNF system, which exerts a trophic action on dopaminergic neurones in the ventral midbrain contributing to schizophrenia.

Ketamine (Ket) is a clinically used dissociative anaesthetic with pharmacological effects similar to phencyclidine (PCP). It was suggested that phencyclidine and Ket may not produce a selective hypoglutamatergic state, but more likely produce a non-selective multi-system neurochemical perturbation *via* direct and indirect effects [28]. Recently, we reported on Ket-induced changes in rat behaviour [29]. Two weeks after completion of subchronic treatment with 30 mg kg^{-1} Ket, latent inhibition was found to be disrupted and social interaction was altered. Binding studies revealed a decreased glutamate receptor binding in the frontal cortex that fits in well with the enhanced locomotor activity after MK-801 injection. Histopathological investigations demonstrated changes in the distribution patterns of parvalbumin, nNOS, and cFos immunopositive and NADPHd reactive cells in hippocampal areas similar to those found in human schizophrenia [30]. It was suggested that subchronic treatment with subanaesthetic doses of Ket induce schizophrenia-related alterations, which might be a useful model in the study of schizophrenia. To further validate this model, we measured two weeks after completion of subchronic pretreatment with Ket the mRNA expression of neurotrophic factors NGF, NT-3, and BDNF and their receptors TrkA, TrkB, and TrkC, respectively, which are involved in the maintenance of neurone viability and neuronal communication in different brain areas of rats.

RESULTS AND DISCUSSION

In the present study we investigated mRNA expression of neurotrophic factors and their high affinity receptors in different brain regions. We examined the FC, the striatum (Str), the dorsal Hip, the thalamus/hypothalamus (THH), and the cerebellum (Cer) of Ket-pretreated rats after subchronic treatment with the neuroleptic drug risperidone (Ris) compared with controls receiving saline (sal). We found significant interactions of pretreatment and treatment for NGF in THH and Cer (Tab. 1) and for BDNF and TrkB in Hip (Fig. **1**). Treatment effects were shown for BDNF and NT-3 in Cer (Tab. 1). Pretreatment results significant differences in the mRNA expression for NGF in Hip, Str and THH, for BDNF in FC, Hip and Cer, for NT-3 and TrkA in all investigated regions, for TrkB and TrkC in Str, THH and Cer and TrkB also in Hip (Table **1**, Fig. **1**).

1. Frontal cortex (Table **1**)

Two-way ANOVA revealed that pretreatment (Ket and sal) did not affect NGF, TrkB and TrkC levels. There was a significant effect on BDNF ($F\ 1, 23 = 15.28$, $p = 0.001$), NT-3 ($F\ 1, 23 = 144.4$, $p < 0.001$) and TrkA ($F\ 1, 23 = 249.32$, $p < 0.001$).

There was no effect of treatment (Ris and sal) in any neurotrophic factor measured and there was no significant pretreatment x treatment interaction.

One-way ANOVA revealed significant differences in:

BDNF: Ris did not change BDNF in the control group. The levels in Ket/sal and Ket/Ris are significantly higher compared with the sal/sal and sal/Ris group.

NT-3: Ris treatment did not affect the control group. All the Ket-pretreated groups had significantly lower NT-3 levels than the controls.

TrkA: Ris treatment did not alter TrkA levels in the sal-pretreated groups and in the Ket-pretreated animals TrkA levels were significantly lower compared with the sal-injected control animals.

2. Hippocampus

Two-way ANOVA showed that pretreatment (Ket and sal) had no effect on TrkC. There was a significant effect on NGF ($F\ 1, 23 = 28.58$, $p < 0.001$), BDNF ($F\ 1, 23 = 4.93$, $p = 0.038$), NT-3 ($F\ 1, 23 = 16.87$, $p = 0.001$), TrkB ($F\ 1, 23 = 99.45$, $p < 0.001$) and TrkA ($F\ 1, 23 = 70.53$, $p < 0.001$).

Treatment (Ris and sal) had no influence on NGF, and TrkA. There was a significant effect on BDNF ($F\ 1, 23 = 9.01$, $p = 0.007$), NT-3 ($F\ 1, 23 = 7.6$, $p = 0.012$), TrkB ($F\ 1, 23 = 33.38$, $p < 0.001$) and TrkC ($F\ 1, 23 = 0.017$).

There was a significant pretreatment x treatment interaction at the BDNF ($F\ 1, 23 = 9.38$, $p = 0.006$) and TrkB ($F\ 1, 23 = 10.32$, $p = 0.004$) NT-3 level ($F\ 1, 23 = 5.69$, $p = 0.027$).

One-way ANOVA revealed significant differences in:

NGF: In the control group, treatment with Ris did not significantly affect NGF. Ket/sal and Ket/Ris had significantly lower NGF levels compared with sal/sal and Ris/sal.

BDNF: The BDNF level was significantly enhanced in animals from the Ket/sal group compared with sal/sal and sal/Ris. Regardless of the tendency, treatment with Ris had no significant effect in the control group, but it reduced BDNF in Ket/Ris compared with Ket/sal suggesting restorative action.

NT-3: The NT-3 level in the Ket/sal group is significantly higher compared with sal/sal, sal/Ris, and Ket/Ris. There was no difference between sal/sal and Ket/Ris.

TrkA: Ris increased TrkA in the control group dramatically. Compared with sal/sal, TrkA is increased in the Ket/sal group. In the Ket/Ris animals, TrkA is higher than in the sal/sal control group but lower than in Ket/sal.

TrkB: Animals from the sal/sal and sal/Ris groups had significantly higher TrkB levels compared with animals from the Ket groups. Moreover, the difference between Ket/sal and Ket/Ris is significant.

Alterations in the NT-3 level following subchronic pretreatment with Ket were ameliorated with Ris treatment.

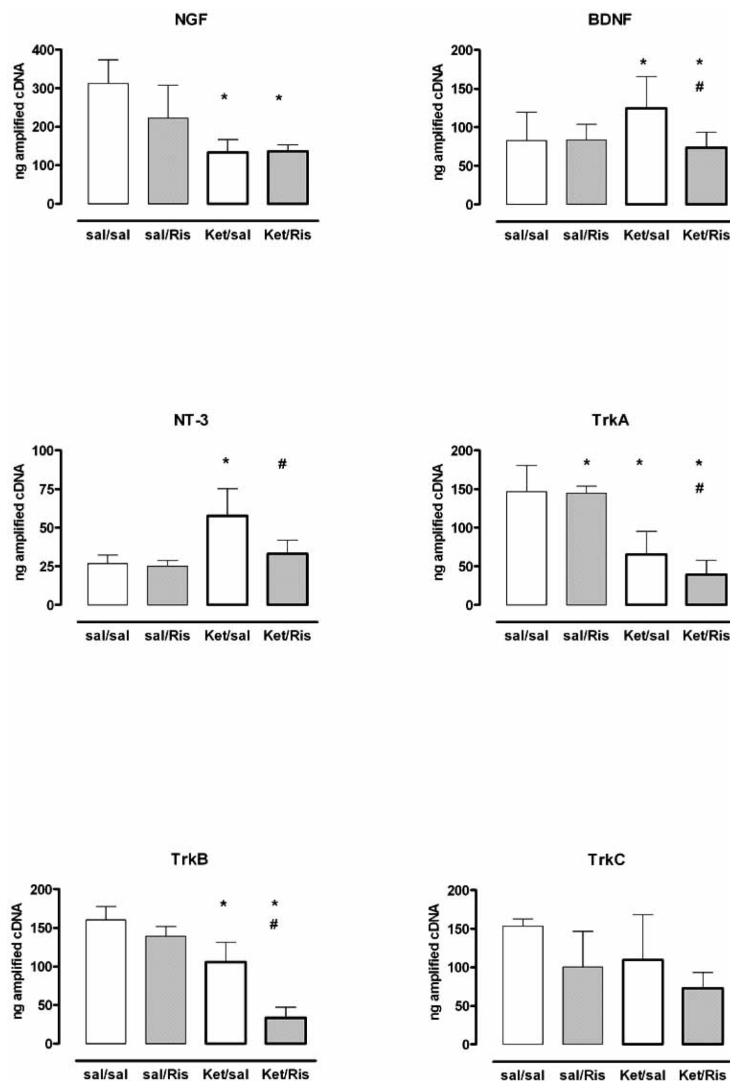

Fig. (1). Effects of Risperidone (Ris) on NGF, BDNF, NT-3, TrkA, TrkB and TrkC cDNA levels in the Hippocampus of sham (sal) and Ketamine (Ket) pretreated rats. Mean ± SEM. 6 animals per group were used.

* $p < 0.05$ *vs*. sal/sal.

3. Striatum (Table **1**).

Two-way ANOVA revealed that with the exception of BDNF, in Str all parameters were affected by pretreatment (Ket and sal). There was a significant effect on NGF (F 1, 23 = 17.38, $p < 0.001$), NT-3 (F 1, 23 = 118.9, $p < 0.001$), TrkA (F 1, 23 = 4.96, $p = 0.038$), TrkB (F 1, 23 = 7.24, $p = 0.014$) and TrkC (F 1, 23 = 54.96, $p < 0.001$). Neither treatment and nor pretreatment x treatment interaction had any effect.

One-way ANOVA revealed significant differences in:

NGF: NGF in the sal/sal group was significantly higher compared with Ket/sal and Ket/Ris.

NT-3: In the sal/sal and sal/Ris groups NT-3 was higher than in the Ket/sal and Ket/Ris groups.

TrkA: Due to large deviations between the 4 groups the differences remain insignificant.

TrkB: In the sal/sal and sal/Ris groups TrkB was higher than in the Ket/sal and Ket/Ris groups.

TrkC: Ris treatment resulted in a significantly higher TrkC level in the sal/Ris group compared with sal/sal. In Str of Ket/sal and Ket/Ris treated rats, we measured higher TrkC levels compared with controls. Finally, TrkC was elevated in sal/Ris compared with Ket/sal and Ket/Ris.

4. Thalamus/Hypothalamus (Table **1**)

Two-way ANOVA revealed that pretreatment Ket did not affect BDNF levels in this structure. There was a significant effect on NGF (F 1, 23 = 34.43, $p < 0.001$), NT-3 (F 1, 23 = 17.81, $p < 0.001$), TrkA (F 1, 23 = 24.18, $p < 0.001$), TrkB (F 1, 23 = 10.58, $p = 0.004$), and TrkC (F 1, 23 = 25.77, $p < 0.001$).

Treatment (Ris and sal) did not affect BDNF, NT-3, TrkA, TrkB and TrkC. There was a significant effect on

Table 1. Effects of Risperidone (Ris) on NGF, BDNF, NT-3, TrkA, TrkB and TrkC cDNA Levels (ng Amplified cDNA) in the Frontal Cortex, Striatum, Thalamus/Hypothalamus and Cerebellum of Sham (sal) and Ketamine (Ket) Pretreated Rats. Mean ± SEM. 6 Animals per Group were used. ANOVA, * p < 0.05 *vs.* sal/sal (Post hoc Bonferroni Test), n.s. = not significant

Structure	sal/sal	Ket/sal	sal/Ris	Ket/Ris	F
Frontal cortex					
NGF	101.0 ± 23.0	152.5 ± 28.4	99.8 ± 35.2	136.5 ± 17.6	n.s.
BDNF	40.5 ± 8.2	106.0 ± 32.7*	42.3 ± 8.5	73.3 ± 42.9*	F 3, 20 = 6.26, p = 0.04
NT3	20.7 ± 3.6	16.2 ± 2.2	19.6 ± 3.4	14.7 ± 1.0	F 3, 20 = 48.52, p < 0.001
TrkA	6.8 ± 0.6	3.8 ± 0.1	6.6 ± 0.5	3.8 ± 0.2	F 3, 20 = 33.39, p < 0.001
TrkB	95.7 ± 28.0	126.6 ± 48.9	68.5 ± 16.1	115.7 ± 25.2	n.s.
TrkC	108.2 ± 27.7		103.2 ± 25.5	87.9 ± 33.2	n.s.
Striatum					
NGF	15.4 ± 4.9	8.7 ± 1.9*	14.3 ± 3.6	8.6 ± 1.6*	F 3, 20 = 5.88, p = 0.05
BDNF	8.5 ± 0.3	7.8 ± 1.3	8.4 ± 0.4	6.7 ± 0.4	n.s.
NT3	8.4 ± 0.3	4.1 ± 0.4*	5.9 ± 0.7	3.9 ± 0.2	F 3, 20 = 397.2, p < 0.001
TrkA	35.9 ± 35.3	3.3 ± 0.9	98.5 ± 14.4	6.3 ± 5.2	n.s.
TrkB	41.4 ± 34.0	16.6 ± 5.0*	56.8 ± 27.7	27.0 ± 10.7*	F 3, 20 = 3.24, p = 0.048
TrkC	64.1 ± 21.1	8.3 ± 1.3	61.4 ± 26.4	9.9 ± 2.9	F 3, 20 = 18.99, p < 0.001
Thala-mus/Hypothalamus					
NGF	65.0 ± 34.1	32.2 ± 27.9	123.2 *± 18.7	22.6 ± 17.1	F 3, 20 = 15.9, p < 0.001
BDNF	52.5 ± 17.9	48.0 ± 28.8	65.8 ± 7.7	76.8 ± 22.9	n.s.
NT3	11.7 ± 0.7	8.7 ± 1.4	11.7 ± 1.1	10.3 ± 1.3	F 3, 20 = 7.64, p = 0.001
TrkA	73.8 ± 51.3	16.0 ± 8.4*	100.6 ± 33.7	19.2 ± 13.3*	F 3, 20 = 8.63, p = 0.001
TrkB	155.0 ± 18.8	132 ± 14.7*	165.9 ± 20.5	114.5 ± 40.2*	F 3, 20 = 4.06, p = 0.021
TrkC	151.7 ± 34.3	83.6 ± 26.2*	167.6 ± 14.6	114.4 ± 28.0*	F 3, 20 = 9.97, p < 0.001
Cerebellum					
NGF	14.9 ± 6.1	36.9 ± 23.7*	96.5 ± 23.6*	60.4 ± 47.6	F 3, 20 = 7.13, p < 0.002
BDNF	20.6 ± 5.1	41.6 ± 11.8*	31.1 ± 7.7	49.0 ±11.2*	F 3, 20 = 8.73, p < 0.001
NT3	22.0 ± 4.0	7.0 ± 1.3*	27.8 ± 4.8	11.8 ±6.0*	F 3, 20 = 23.18, p < 0.001
TrkA	6.2 ± 0.7	3.9 ± 0.1*	6.7 ± 1.7	4.2 ± 0.3*	F 3, 20 = 11.04, p < 0.001
TrkB	25.6 ± 12.9	64.1 ± 17.1*	28.1 ± 8.4	72.5 ± 26.5*	F 3, 20 = 9.1, p < 0.001
TrkC	49.3 ± 20.2	69.7 ± 26.9	73.7 ± 33.6	118.0 ± 30.1	F 3, 20 = 5.27, p = 0.008

NGF ($F_{1, 23} = 4.57$, $p = 0.045$). We found a significant pretreatment x treatment interaction in NGF ($F_{1, 23} = 8.91$, $p = 0.007$), but not in BDNF, NT-3, TrkA, or TrkC.

One-way ANOVA revealed significant differences in:

NGF: In the sal/Ris group we measured higher NGF levels compared with all the other groups. There was no difference between sal/sal and Ket/sal and Ket/Ris, respectively.

NT-3: In the sal/sal and sal/Ris group significantly higher levels were found compared with Ket/sal.

TrkA: In the sal/sal and sal/Ris treated animals, we found significantly higher levels of TrkA than in the Ket/sal and Ket/Ris group.

TrkB: In the sal/sal and sal/Ris group, significantly higher levels were found compared with the Ket treated animals. The difference between sal/Ris and Ket/Ris is significant.

TrkC: In the sal/sal and sal/Ris treated animals, we found significantly higher levels of TrkC than in the Ket/sal and Ket/Ris group.

Alterations in the NT-3 level following subchronic pretreatment with Ket were ameliorated with Ris treatment.

5. Cerebellum (Table **1**)

Two-way ANOVA revealed that with the exception of NGF, pretreatment (Ket and sal) had a significant effect on BDNF (F 1, 23 =21.56, $p < 0.001$), NT-3 (F 1, 23 = 62.15, $p < 0.05$), TrkA (F 1, 23 = 32.2, $p < 0.001$), TrkB (F 1, 23 = 27.916, $p < 0.001$), and TrkC (F 1, 23 = 6.6, $p = 0.018$).

Treatment (Ris and sal) changed levels of NGF (F 1, 23 = 16.17, $p = 0.001$), BDNF (F 1, 23 = 4.49, $p = 0.047$), NT-3 (F 1, 23 = 7.34, $p = 0.013$), and TrkC (F 1, 23 = 8.3, $p = 0.009$), but not TrkA and TrkB. There was a significant pretreatment x treatment interaction in NGF (F 1, 23 = 4.93, $p = 0.038$) but not in the other parameters evaluated.

One-way ANOVA revealed significant differences in:

NGF: In comparison to sal/sal and Ket/sal, the NGF level was significantly elevated in the sal/Ris group.

BDNF: BDNF is significantly higher in the Ket/sal and Ket/Ris group compared with sal/sal and there is a significant difference between sal/Ris and Ket/Ris.

NT-3: In the sal/sal and sal/Ris, NT-3 is significantly higher compared with Ket/sal and Ket/Ris.

TrkA: In the sal/sal and sal/Ris, TrkA is significantly higher compared with Ket/sal and Ket/Ris.

TrkB: In the sal/sal and sal/Ris, TrkB is significantly higher compared with Ket/sal and Ket/Ris.

TrkC: There was a significant difference between sal/sal and Ket/Ris.

Subchronic pretreatment with the non-competitive NMDA antagonists resulted in region-specific alterations in the mRNA level of different neurotrophic factors. Subchronic treatment with the atypical antipsychotic drug Ris normalised BDNF mRNA levels in the hippocampus. This might underline the potency of the Ket model in preclinical schizophrenia research.

It was hypothesised that neuronal malformation in connection with genetic and environmental conditions is one of the basic factors contributing to schizophrenia. This hypothesis would explain effects of pathogenic noxae such as viral infections, maternal malnutrition, pre- and perinatal hypoxia, obstetric complication, excitotoxins, alcohol or other drug intake, stress etc. during specific stages of brain development. There is evidence that neurodevelopmentally derived misconnections in the cortico-cerebellar-thalamic-cortical circuit (CCTCC) contribute to cognitive dysmetria which is considered to be the fundamental deficit in schizophrenia [31]. Since neuronal plasticity persists into later stages of ontogenesis, such noxae might also modulate neuronal circuits located in different areas in the adult brain resulting in functional asynchrony. Neurotrophic factors such as NGF, BDNF, and NT-3 have a decisive role in processes of neurodevelopment, neuronal plasticity and neuronal survival. Moreover, BDNF was reported to modulate the release of dopamine in the the mesolimbic system [32]. Such a modulatory role would explain a significant negative correlation between BDNF-like immunoreactivity and PANSS negative subscore in patients treated with both atypical and typical neuroleptics [20]. For a better understanding of dynamic changes within the brain with special regard to the CCTCC, we investigated neurotrophic factors in the frontal cortex, striatum, hippocampus, cerebellum, and thalamus/hypothalamus of rats subchronically pretreated with Ket which results in the occurrence of schizophrenia-related alterations in behaviour, neurotransmission and neuromorphology [29, 30].

Studies in animal models suggested that particularly NGF and BDNF might be implicated in mechanisms leading to a condition associated with schizophrenia-like behaviour. Interestingly, subchronic pretreatment with Ket resulted in reduced levels of NGF in Hip, Str, and THH, whereas BDNF was upregulated in FC, in the Hip and, in tendency in Cer. It might be hypothesised that region-specific action of neurotrophic factors contributes to the maldevelopment responsible for schizophrenia.

As shown in Table **1** and Fig. (**1**), Ket pretreatment did also result in reduced NGF levels in the Hip, Str, and THH. This correlates well with clinical findings showing reduced NGF plasma levels in schizophrenics [11]. NGF is considered to have a higher affinity for TrkA [33, 34]. Thus, alterations at the receptor levels following NGF reduction seem to be plausible. However, further clarification is needed to explain the different TrkA regulations, i.e. the increased level in the Hip of Ket/sal treated (Fig. **1**) animals whereas TrkA levels are decreased in Str and THH in the same animals (Table **1**).

The neonatal lesion of the hippocampus represents a putative developmental in schizophrenia research. Eighty-four days after the lesion, suppressed BDNF mRNA expression occurred in the gyrus dentatus [35] and in the cingulate cortex [24]. A similar trend was found in the prefrontal cortex [23]. This was explained in terms of DNA damage in response to neonatal insult and dramatic effects of the lesion on synaptogenesis and neuronal development in other parts of the hippocampal formation [23]. Subchronic treatment with Ket did also alter BDNF levels specifically, i.e. increased levels in FC, Hip, and Cer (Table **1**, Fig. **1**). Obviously, regulation of both neurotrophic factors is diametrically opposed. This might result in imbalances in receptor expression and impairment of signal transduction associated with schizophrenia. With the exception of FC, changes in BDNF levels are closely associated with alterations in TrkB, which is considered the signalling receptor of BDNF [33, 34].

Subchronic Ket treatment resulted in increased levels of BNDF in the Hip whereas neonatal lesion of the ventral Hip led to decreased BDNF-mRNA expression [35]. These results are not necessarily contradictory. This discrepancy might reflect differences in the inducing agent (lesion *vs*. Ket), time (neonatal *vs*. adult) and locus for investigation (gyrus dentatus *vs*. dorsal part of the Hip). Typical topographic patterns of BDNF mRNA were found after systemic application of MK-801. This substance also acts as an antagonist of NMDA receptors resulting in psychotomimetic

effects. BDNF mRNA was decreased in the Hip and in the superficial layers of the cerebral cortex, but in middle layers of the cerebral cortex and the midline thalamic nuclei BDNF mRNA levels were markedly increased [36]. This would underline the hypothesis that imbalances in regulatory processes with special reference to hippocampal circuits might contribute to schizophrenia. It was speculated that the key change in hippocampal pathology is probably an alteration in the precise organisation and functioning of neural circuits within the Hip, and which connect it with other structures [37]. Mossy fiber sprouting induced by chronic electroconvulsive seizures increase BDNF expression in the hippocampus. This is reduced by ketamine pretreatment [38].

Clinical observations demonstrate that therapeutic effects of antipsychotic drugs are not observed until 2 – 3 weeks following administration. This suggests that clinical effectiveness of these substances is not solely due to action at neurotransmitter receptors, but also includes alterations in gene expression and protein synthesis [39]. Both typical and atypical antipsychotic drugs were reported to alter in different ways the regional brain levels of NGF and BDNF [40]. Quantitative analysis revealed that the typical antipsychotic drug haloperidol down-regulated BDNF mRNA expression in both CA1 and dentate gyrus regions compared with vehicle control. In contrast, the atypical antipsychotic agents clozapine and olanzapine up-regulated BDNF mRNA expression in the rat hippocampus and its subfields compared with their respective controls [39, 41]. However, Ris which is also considered an atypical antipsychotic drug was found to down-regulate hippocampal BDNF mRNA [17, 41]. This is not conflicting with the results obtained in our investigation (Fig. **1**), since others used higher doses of Ris [41] or long-term oral treatment *via* food pellets [17].

Behavioural observations have shown that subchronic treatment with Ris normalised schizophrenia-related alterations in social behaviour in Ket-pretreated rats [42]. The same dose, i.e. 0.2 mg kg^{-1} was used in the present experiments. In sal-pretreated control rats Ris had no effect on any neurotrophic factor measured in FC and Str (Table **1**). In the Hip, BDNF was elevated and in THH and Cer NGF levels were increased (Table **1**, Fig. **1**). Significant effects in Ket-pretreated rats were only found in the Hip (Fig. **1**). Increased levels of BDNF in Ket/sal rats were lowered. This is in line with results obtained in rats after neonatal ventral hippocampal lesion. It was concluded that antipsychotics down-regulate BDNF mRNA although there therapeutic properties are not mediated *via* this neurotrophin [23, 43]. A similar effect was found in NT-3 and its primary receptor TrkC [33, 34]. Only in the Hip was there a significant pretreatment (i.e. sal and Ket) x treatment (i.e. sal and Ris) interaction in terms of normalisation (Fig. **1**). It was suggested that an alteration in the precise organisation and functioning of neural circuits within the Hip contributes to schizophrenia pathology [37]. BDNF might contribute to these plastic-adaptive alterations which is also underlined by the fact that neurogenesis is enhanced in the hippocampal subgranular zone in rats subchronically injected with Ket [44]. It is debatable whether higher doses of Ris would interfere with BDNF mRNA expression in the other brain regions belonging to the CCTCC. In the study presented a dose was used that was shown to normalize altered social behavior [42]. Clinical studies revealed that lower doses of Ris are as effective for the treatment of positive and negative symptoms compared with administered higher doses [45].

Taken together, subchronic application of Ket results in changes in neurotrophin and neurotrophin receptor mRNA expression in different brain areas associated with schizophrenia. This further underlines the validity of the model. Moreover, the antipsychotic drug Ris that was found to normalise behavioural abnormalities did also balance BDNF levels in the Hip. The data might also suggest a prominent role of BDNF in the Hip for processes related to schizophrenia.

EXPERIMENTAL SECTION

The experiments were performed in accordance with the regulations of the National Act on the Use of Experimental Animals and E.U. guidelines.

Animals

Animals were naive male Sprague-Dawley rats (Shoe: SPRD, DIMED Schönwalde, Germany). The rats were kept under controlled laboratory conditions with a light/dark cycle 12:12 (lights on at 06.00 a.m.), temperature 20 ± 2°C, and air humidity 55 - 60%. They had free access too commercial rat pellets (Altromin 1326) and tap water. The animals were housed in groups of 5 in Macrolon IV cages. At the beginning of the injection period, the rats aged 8 weeks old.

Drugs

The Ket-pretreated animals were injected with 30 mg kg^{-1}. Ket (CU Chemie Uetikon GmbH, Germany) intraperitoneally (ip) at a volume of 1 ml/100 g body weight daily for 5 consecutive days. Control animals received isotonic saline (sal) at corresponding times [29]. To study the effects of the potent neuroleptic drug Risperidone (Janssen Biotech N.V., Olen, Belgium; Ris, 0.2 mg kg^{-1}, solved in saline adding three drops of Tween 80) the animals were divided into 4 experimental groups (sal/sal, sal/Ris, Ket/sal, Ket/Ris) at random immediately after Ket pretreatment completion. The dose administered was found to normalise altered social behaviour in Ket-pretreated rats [42]. Ris injections were given ip daily between 08:00 – 10:00 a.m. over a period of 10 days as described above. Twenty-four hours after the final injection, the animals were sacrificed. The substances were expected to have cleared by this time.

Tissue Preparation

Six animals were used per treatment group. The rats were killed by stunning and decapitation. The brains were rapidly removed from the cranium, placed on an ice-chilled petri dish and they were dissected in the following areas: frontal cortex (FC), striatum (Str), dorsal hippocampus (Hip), thalamus/hypothalamus (THH), and cerebellum (Cer). All the specimens were frozen on dry ice and stored at –80°C for further analysis.

mRNA Determination

The protocol for mRNA determination has been previously described in detail [46, 47]. Briefly, total cellular RNA

was isolated according to the TRIzol-protocol and transcribed by a reverse transcriptase (RT) into a cDNA copy: One ml TRIzol (Invitrogen, Carlsbad, CA, USA) was added to the frozen material in cryotubes and completely homogenised. After incubation 0.2ml chloroform was added and centrifuged. The aqueous phase with RNA was mixed with 0.5ml isopropyl alcohol. After centrifugation the RNA containing pellet was washed in 75% ethanol and pelleted at 7,500xg. The use of DEPC-treated nuclease-free water and sterilisation of all instruments and materials ensured absence of nucleases. The structural integrity of the RNA was confirmed by electrophoresis in 1% agarose-formaldehyde gels. RNA amount and purity were measured in an UV/VIS spectrophotometer and adjusted to 1µg RNA/µl. The cDNA was synthesised by transcription of 4µg RNA with M-MLV RT RNase H minus (Promega, Madison, WI, USA) using oligo-dT primer in a thermal cycler (Unoblock, Biometra, Göttingen). Primer (1µl, 0.5µg/ml) and template (4µl) were incubated at 4°C before adding all components (0.5mM dNTP, 20U RNase inhibitor, volume adjusted to 40µl) except RT and then further incubated for additional 5 min. Then, 1µl (200U/µl) of RT was added and cDNA-synthesis started for 60 min at 42°C. Translation was finished by heating (70°C). A positive control was run according to the manufacturer's protocol, negative controls were transcribed either omitting RT or RNA and amplified later within the PCR.

Amplification of first-strand cDNA-copy was performed with Taq DNA polymerase (Promega, Madison, WI, USA) with the following primers: NGF sense TCC AGG TGC ATA GCG TAA TG, antisense CTC CGG TGA GTC CTG TTG AA (273-645, NGF last exon); BDNF sense AGC CTC CTC TGC TCT TTC TG, antisense CTT TTG TCT ATG CCC CTG CA (386-664); NT-3 sense TTT CTC GCT TAT CTC CGT GG, antisense GGC AGG GTG CTC TGG TAA TT (115-263); TrkA sense CAC CAG TGA TCT CAA CAA GA, antisense GTT GAA CTC AAA AGG GTT GT (829-1228); TrkB sense CCA CTA GGA TTT GGT GTA CC, antisense CCA CTG TCA TCC GAT GAA AT (783-1446); TrkC sense GCA TCA ACA TCA CGG ACA TC, antisense GAG CCA GAG CCA TTA CAA GT (209-707).

Each sample was double analysed. The protocol started with initial denaturation for 2 min at 94°C and subsequent application of Taq polymerase. The amplification was performed as follows: denaturation at 94°C for 40s, annealing at 55°C (TrkB, TrkA) and 60°C (NGF, BDNF, NT-3 and TrkC) for 1 min and amplification at 72°C for 1 min. This profile was repeated 28 to 33 times (NGF: 31, BDNF: 29, NT-3: 28, TrkA: 30, TrkB: 28, TrkC: 27). A final elongation was run for 15 min at 72°C and stopped at 4°C. We used a single protocol without nesting to eliminate false positive and false negative results. The assays were performed in 0.2 ml tubes in a total volume of 25 µl containing cDNA made from 2 µg of total RNA with 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatine (w/v), 2.5 units Taq-polymerase, 0.2 µM of each primer and 200µM of each dNTP. The heating lid of the thermocycler was adjusted to 110°C during the whole PCR. A full negative control was run to detect any possible transfer of previous PCR products and contamination of mastermix. Negative RT-PCR control omitting RT provided information on potential genomic DNA amplification. A positive control was also run to analyse any failures during PCR process.

RT-PCR products were analysed by electrophoresis (Serva BlueLine, Boehringer-Ingelheim, Germany). Samples were transferred to a 1.25% agarose gel (Boehringer Mannheim, Germany). Gels were run for 1h at 80 mV. The ethidiumbromide-stained gels were digitized and detected by using video densitometry software (E.A.S.Y. Win32 (Herolab, Wiesloch, Germany). For quantitation, a molecular mass ladder marker (GibcoBRL, Eggenstein, Germany) was used. The marker was run within the gels and consisted of bands of 10, 20, 40, 80, 120 and 200 ng dsDNA. A representative sample was given in Fig. **2**. The densities were compared to PCR products and calculated with the analysis software E.A.S.Y. Win 32 (Herolab Wiesloch, Germany). A beta-actin standard had been run in advance for checking the viability and standardisation of the PCR.

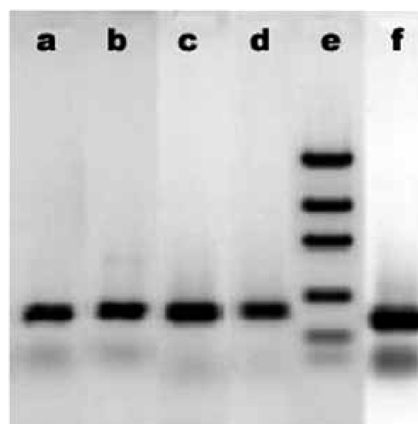

Fig. (2). cDNA fragments of BDNF from the hippocampus amplified by RT-PCR and electrophoretically isolated; lanes: **a** sham **b** Risperidone treated **c** Ketamine pretreated **d** Risperidone after Ketamine pretreatment **e** marker DNA mass ladder with equimolar DNA fragments containing 10, 20, 40, 80, 120 and 200 ng of DNA (Life Technologies, Rockville, USA), respectively, **f** cDNA fragments of beta-actin.

Data Analysis

The statistical analysis was based on Two-way Analysis of Variance with pretreatment (Ket *vs.* sal) and treatment (Ris *vs.* sal) being the independent variables followed by one-way ANOVA. Post hoc Bonferroni test was used (SPSS+ software). The significance threshold was set at 0.05.

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