VIRAL INFECTION, GLUTAMATERGIC DEFICIT AND BEHAVIORAL CHANGES IN ANIMAL MODEL OF SCHIZOPHRENIA

VIROVÁ INFEKCE, GLUTAMÁTERGNÍ DEFICIT A ZMĚNY CHOVÁNÍ V ANIMÁLNÍM MODELU SCHIZOFRENIE

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Summary

Retroviruses can disrupt brain development, cause neuronal death and induce behavioural changes and therefore they have been proposed to play a role in the etiology of schizophrenia. Quinolinic acid (QUIN), released from retrovirus-infected brain macrophages and microglia, might be responsible, at least in part, for most of the proposed alterations. Intracerebroventricular infusion of QUIN to rat pups did caused a neuronal damage and evoked a subsequent diminution of the specific membrane binding of glutamate and changed their exploratory and acoustic startle activities in early adulthood. The changes were modified (potentiated) by increased levels of brain dopamine and inhibited by haloperidol and clozapine. Our present data suggest that increased levels of QUIN in neonatal rat brain can induce changes in the brain development and function which exhibit some similarities to human schizophrenia.

Key words: schizophrenia, animal model, quinolinic acid, NMDA receptor, dopamine, haloperidol, clozapine, acoustic startle reaction, prepulse inhibition, open field test

Souhrn

Retroviry mohou narušit vývoj mozku, způsobit zánik neuronů a vést ke změněnému chování, z čehož se v poslední době vyvozuje jejich možná úloha v etiologii schizofrenie. Kyselina chinolinová (QUIN), neurotoxický metabolit tryptofanu tvořený a secernovaný infikovanými mozkovými makrofágy a mikroglií, by se mohl spolupodílet na těchto změnách. Intracerebroventrikulární infuze QUIN mláďatům potkana skutečně způsobila nejen neuronální poškození, ale i následné snížení vazby glutamátu do membrán mozkových buněk a změny v explorační a úlekové aktivitě těchto zvířat v jejich časné dospělosti. Tyto změny byly dále modifikovány zvýšením hladiny dopaminu v mozku dospívajících zvířat a potlačeny haloperidolem nebo klozapinem. Tyto údaje ukazují, že zvýšené hladiny QUIN v neonatálním mozku potkana mohou způsobit změny ve vývoji mozku a jeho funkcích, které vykazují některé podobnosti se schizofrenií člověka.

Klíčová slova: schizofrenie, animální model, kyselina chinolinová, NMDA receptor, dopamin, haloperidol, klozapin, akustická úleková reakce, prepulzní inhibice, test volného pole

The neurodevelopmental hypothesis of schizophrenia is based on a proposition that early abnormal brain development results from an exposure to adverse environmental factors associated with prenatal/perinatal viral infections, like influenza (Mednick et al., 1988; Izumo et al., 1999), Borna disease virus (Iwahashi et al., 1998) or retroviruses (Crow, 1984; Hart et al., 1999). Perinatal exposure of rodents to corresponding infectious agents (and/or to their

toxins) demonstrated close resemblance of functional and neuropathologic abnormalities to human schizophrenic psychosis (Cotter et al., 1995; Horning et al., 1999; Hill et al., 1993; Pittaluga et al., 2001). Retroviral nucleotide sequences were found in the cerebrospinal fluid (and frontal cortex) of 29 % of individuals with acute onset of schizophrenia and in 5% of patients in later stages of the disorder (Karlsson et al., 2001). Neuronal apoptosis/necrosis and disturbances in neuroblast migration with subsequent neuronal disarray may result from viral infection *per se* or from virus-derived toxic mediators, such as the surface glycoprotein 120 (gp120) and quinolinic acid (QUIN) (Epstein and Gendelman 1993). Gp120 may act through toxic proinflammatory cytokines, namely IL-1β and TNF-α (Ilyin and Plata-Salamán, 1997) but apparently not through an agonist of the *N*-methyl-D-aspartate (NMDA)-type of glutamate receptors. In contrast, QUIN may interact with a subpopulation of the NMDA receptors and induce a neuronal damage/loss in animal model (Beskid, 1994) and cerebral brain atrophy in children (Brouwers et al., 1993).

QUIN, an endogenous metabolite of tryptophan in macrophages/microglia, is a cytokine-like molecule specific for immune system, with an ability to preferentially activate NMDA heteromers of NR1/NR2B subunit composition. The subunit NR2B and universal NR1 subunit are widely expressed in the whole neonatal rat brain whereas in adulthood the expression of NR2B subunit is mainly limited to the hippocampus, neocortex and neostriatum. About a week after a viral inoculation there is a dramatic overproduction of QUIN accompanied by the onset of neurodegeneration in the rodent brain (Eastman et al., 1994). As the susceptibility to QUIN-induced neuronal damage is particularly high in the immature rat brain with peak susceptibility to QUIN-induced neurotoxicity occurring between postnatal days 7 and 14 (Trescher et al., 1994), in our neurodevelopmental (animal) model of schizophrenia, we stereotaxically infused QUIN into each lateral cerebral ventricles in a dose of 250 nmol/ventricle on postnatal day 12 (Lisý et al., 1994). Male rats were distributed at random into three groups - intact, sham-operated (control) and QUIN-lesioned animals. Neither sham-operated nor QUIN-lesioned animals were separated from their mothers for more than 3 h to avoid negative effects of early maternal deprivation (Ellenbroek and Cools, 2002). All procedures involving animal experimentation followed regulations of National Committee for the Care and Use of Laboratory Animals.

Schizophrenia has been associated simultaneously with both hypoactivity of glutamatergic cortical elements and hyperactivity of mesostriatal and mesocortical dopaminergic system (Konradi and Heckers 2003). In animal model QUIN-induced abnormal neuronal development was followed by decreased glutamate receptor binding to synaptic

membranes isolated from various cortical and subcortical rat brain regions in early adulthood. The decrease was not found 4 days after the intracerebroventricular (i.c.v.) infusion of QUIN to 12-day-old rats (Lisý et al., 1994) but was evident with a delay of more than 5 weeks. The administration of GBR 12909 (10 mg/kg b.w., i.p.), a potent and highly selective competitive inhibitor of dopamine (DA) uptake, to post-pubertal rats on postnatal day 50 served as a model of the schizophrenia-like subcortical dysregulation of dopaminergic neurotransmission. Exacerbation of psychotic (behavioural) symptoms in response to resulting mesolimbic DA-ergic hyperactivity was studied in "observation I" whereas diminution of the behavioural activities by typical (haloperidol; 0.1 mg HAL/kg b.w., s.c) and atypical (clozapine; 10 mg CLZ/kg b.w., s.c) antipsychotics was tested in "observation II".

In the "observation I" all three groups of young adult rat males were in a 5 min "open field test" 30 min after the administration of GBR 12909. The inhibition of dopamine transporter (DAT) resulted in "hyper-dopaminergic state" characterized by locomotor and rearing hyperactivities in the test sessions. Long-term monitoring in a cycle actograph (from 3 p.m. to 7 a.m.) revealed that the DAT inhibition significantly increased horizontal locomotor activity within a period of 120 and 480 min after GBR 12909 administration.

In the "observation II" rats in all groups were pre-treated either with HAL (an antagonist with selectivity for D_2-like receptors which also up-regulates some NMDA receptor heteromers) or CLZ (antipsychotic effect is mediated by occupation of more D_4 than D_2 receptor subtype, by antagonist action at 5-$HT_{2A/2C}$ receptors and by enhancement of NMDA receptor activity) 30 min before they were injected with GBR 12909 and then, after a pause of 30 min, their spontaneous motor activity was tested in a 5 min session of the "open field test". HAL suppressed GBR 12909-induced behaviour but some activities, namely crossing, rearing and floor sniffing were inhibited less effectively in QUIN-treated animals than in control and intact rats. In contrast, CLZ inhibited behavioural activities in all animal groups in parallel and the vertical (rearing) activity and grooming were even absent. However, stereotypic "vertical" head movements were observed. Comparing the action of HAL and CLZ, the atypical antipsychotic seems to normalize the mesolimbic "hyper-dopaminergic state" in QUIN-altered rats more precisely than HAL. In addition, results from another experimental method, evaluating sensorimotor gating (acoustic startle reactivity and PPI) deficient in schizophrenic patients, also showed changes in QUIN group as consequences of brain insult (Šťastný et al., in press).

In conclusion the described neurodevelopmental (animal) model of schizophrenia-like psychosis fulfils three levels of validity:

Face validity (phenomenological similarity) in

- discrete morphological brain lesion;
- diminution of social-contact behavior during prepuberty;
- paroxysmal hyperactivity (hyperlocomotion);
- sensorimotor gating deficits (disruption of PPI).

Construct validity (possible etiology and pathophysiology) is represented by

- prenatal viral exposure (and infection-induced endogenous toxins like QUIN) as environmental factors with etiological and pathophysiological importance;
- this model supports the neurodevelopmental hypothesis of schizophrenia.

Predictive validity (pharmacological manipulations) is based on the ability of antipsychotic drugs to reduce

- existing hypofunction of glutamatergic system;
- a transient hyperfunction of populations of dopaminergic neurons.

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