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DEFICIENCY OF NEURAL CELL ADHESION MOLECULE OR ITS POLYSIALYLATION MODULATES PHARMACOLOGICAL EFFECTS OF THE AMPA RECEPTOR ANTAGONIST NBQX

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Abstract—The neural cell adhesion molecule NCAM and its dynamically regulated posttranslational modification polysialic acid (PSA) are major determinants of cellular interactions during ontogeny. While NCAM in the absence of PSA stabilizes cell–cell interactions, the attachment of the large and polyanionic PSA negatively influences cell adhesion and promotes plasticity. Disease-associated changes in the polysialylation state of NCAM raise the question whether the PSA-NCAM system can affect CNS pharmacology.

Here we investigated the pharmacological effects of the competitive AMPA antagonist NBQX in genetic mouse models either lacking NCAM and PSA (female NCAM knockout mice) or being drastically reduced in the level of PSA expression (female ST8SiaIV knockout mice). Studies were carried out with the respective wildtype littermate controls. In mice lacking NCAM and PSA, NBQX-induced ataxia proved to be more intense as compared with wild-type mice. On both mutant backgrounds, NBQX significantly elevated seizure thresholds during i.v. infusion of the chemoconvulsant pentylenetetrazole.

In summary, the data demonstrate that the PSA-NCAM system impacts AMPA receptor pharmacology under *in vivo* conditions. The fact that comparable effects were observed in NCAM- and ST8SiaIV-knockout mice indicates that this impact is not due to a stabilizing effect of NCAM in the absence of PSA. Thus, disease-related changes in the polysialylation of NCAM are likely to be associated with effects on the efficacy and tolerability of AMPA receptor antagonists. © 2008 Published by Elsevier Ltd on behalf of IBRO.

The neural cell adhesion molecule NCAM is a member of the immunoglobulin superfamily of adhesion molecules present in the CNS (Kiss et al., 2001) and the major carrier of the large negatively charged carbohydrate polysialic acid (PSA) (Kleene and Schachner, 2004). Due to size and charge PSA functions as a biological spacer reducing adhesive effects mediated by its carrier molecule NCAM and other cell surface receptors (Kleene and Schachner, 2004). Consequently the expression of PSA has been correlated with dynamic changes in membrane contacts (Rutishauser et al., 1988; Kiss et al., 2001). Two enzymes are responsible for the formation of PSA, the polysialyltransferases ST8SiaII and ST8SiaIV. The two polysialyltransferases are highly conserved at primary sequence level and are individually able to polysialylate NCAM (for review see Angata and Fukuda, 2003), their expression patterns, however, differ significantly during development and in the adult animal. While ST8SiaII mRNA levels extend the ST8SiaIV levels during ontogeny and early postnatal development, ST8SiaIV is the predominant enzyme in the adult brain. These expression patterns, which were initially identified by Northern blotting and *in situ* hybridization, have been confirmed in mice genetically modified to only express one polysialyltransferase (Eckhardt et al., 2000; Bukalo et al., 2004).

Whereas the polysialylated form of NCAM (PSA-NCAM) is abundant during development, the major NCAM fraction in the adult brain does not carry PSA (Angata and Fukada, 2003). In the adult brain PSA-NCAM is restricted to regions with a high degree of plasticity such as the hippocampus or the olfactory bulb (Kleene and Schachner, 2004).

NCAM can interact in homophilic and heterophilic manner with binding partners in the same (cis) or in neighboring (trans) cell membranes (for review see Kiselyov et al., 2005). Cis interactions of NCAM have been described for L1 (Horstkorte et al., 1993), the fibroblast growth factor receptor (Kiselyov et al., 2005), the glial cell line–derived neurotrophic factor receptor α (Paratcha et al., 2003), and the glucocorticoid receptor (Crossin et al., 1997). In the presence of PSA these NCAM-mediated interactions are altered partly because of the stereo-chemical properties of the carbohydrate unit. On the other hand PSA has been found to mediate autonomous functions. Recent studies demonstrate that PSA modulates the activity of the AMPA as well as the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors in *in vitro* preparations (Vaithianathan et al., 2004; Hammond et al., 2006). These data are of specific interest, because both AMPA and NMDA receptors are involved in activity-dependent plasticity (Shi et al., 1999; Carroll et al., 1999). Therefore PSA-NCAM may function as an important regulator of this plasticity.

Based on the impact of PSA-NCAM on receptor function and on disease-associated changes in the polysialylation state of NCAM, we were asking whether modulatory effects of the PSA-NCAM system on AMPA receptor pharmacology can be demonstrated under *in vivo* conditions. The concurrent use of NCAM knockout mice

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Abbreviations: NCAM, neural cell adhesion molecule; NMDA, *N*-methyl-D-aspartate; PSA, polysialic acid; PTZ, pentylenetetrazole.

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and ST8SiaIV knockout mice, which, in adulthood are dramatically reduced in their PSA-levels, allowed us to address both the impact of NCAM and of PSA on AMPA receptor function. For the ST8SiaIV knockout mice it has been hypothesized that the effect of the reduction of PSA may be partly mediated by a gain of NCAM function (Weinhold et al., 2005). Therefore these mice can be used to study the consequences of a stabilizing effect of NCAM.

Because NCAM represents the major carrier of PSA, NCAM knockout mice not only lack NCAM but also PSA (Cremer et al., 1998). Therefore these mice allow study of the consequences of a complete deficiency of the PSA-NCAM system.

The pharmacological characterization in knockout and wild-type mice comprised a comparison of the anticonvulsant efficacy as well as the behavioral effects of the competitive AMPA antagonist NBQX.

EXPERIMENTAL PROCEDURES

Animals

NCAM-deficient and ST8SiaIV-deficient mice have been described previously by Cremer et al. (1994) and by Eckhardt et al. (2000), respectively. These knockout mice show no apparent differences in phenotype. All analyses were performed on a C57BL/6J background (at least six generations backcrosses) in female animals aged between 5 and 9 months in case of St8SiaIV knockout (n=37) and corresponding wild-type mice (n=21) and aged between 3 and 9 months in case of NCAM knockout (n=26), heterozygous (n=22), and corresponding wild-type mice (n=27). Throughout all experiments investigators were not aware of the genotype. All experiments were approved by the responsible governmental animal review board (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit) and were done in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize pain or discomfort of the animals used and to minimize the animal number.

Compounds

NBQX (used as sodium salt, kindly provided by Novo-Nordisk, Malov, Denmark) and pentylenetetrazole (PTZ) (Sigma Aldrich, Taufkirchen, Germany) were freshly dissolved in saline. A dosage of NBQX was chosen that has slight but not tremendous effects on PTZ thresholds that are likely to exhibit only partial inhibition, and, thus, allows us to detect an impact of genetic deficiencies on the anticonvulsant efficacy. Basis for the selection of the dosage was a study from Löscher and Hönack (1994), in which 80 mg/kg NBQX induced only slight increases in the PTZ thresholds for myoclonic and clonic seizures in male mice of the NMRI outbred strain.

PTZ seizure thresholds

Fifteen minutes following i.p. administration of 80 mg/kg NBQX or following vehicle administration thresholds for PTZ-induced seizures were determined by infusion of a 1% solution of PTZ into the tail vein of unrestricted freely moving mice at a rate of 0.3 ml/min with an infusion pump (Perfusor-E, Braun Melsungen, Melsungen, Germany). The injection cannula was inserted immediately before testing in awake animals. Directly following insertion of the cannula the i.v. PTZ infusion was started and the mouse was released from fixation. Endpoints were first myoclonic twitch, first generalized seizure with loss of righting reflexes, forelimb tonus, and hindlimb tonus. The thresholds were calculated in mg/kg PTZ. PTZ tests were done in all control and experimental groups. Although this is the standard approach for the i.v. PTZ model, we would like to mention that the manipulation of the animal prior to the infusion might impact seizure thresholds. Of course, the procedure was performed in a standardized manner in all animals.

Behavioral effects of NBQX

Behavioral effects induced by i.p. administration of 80 mg/kg NBQX were investigated in wild-type, ST8SiaIV knockout, and NCAM knockout mice.

Time points of behavioral observation were before drug administration and 14 min following NBQX administration. For evaluation of behavioral effects each individual animal was observed for 1 min at each time point in an open field. During this 1-min observation period both ataxia and locomotion were scored.

Ataxia scores were of interest because a muscle relaxant effect of NBQX has been previously described (Turski et al., 1993; Löscher and Hönack, 1994). Ataxia was scored using a validated six-point rating system as described previously (Potschka and Löscher, 1999): 1, slight ataxia in hind legs (tottering of the hindquarters); 2, more pronounced ataxia with dragging of hind legs; 3, further increase of ataxia and more pronounce dragging of hind legs; 4, marked ataxia, animals lose balance during forward locomotion; 5, very marked ataxia with frequent loss of balance during forward locomotion; 6, permanent loss of righting reflexes, but animal still attempts to move forward. Locomotion was scored in the open field using a validated scoring system as described previously (Potschka and Löscher, 1999): −3, intense hypolocomotion; −2, hypolocomotion present; −1, hypolocomotion equivocal; 0, normal locomotion; +1, hyperlocomotion equivocal; +2, hyperlocomotion present; +3, intense hyperlocomotion.

Statistics

Significance of differences in seizure thresholds between knockout and wild-type mice was calculated by ANOVA and Student's t-test for unpaired data. Behavioral data were analyzed by Mann-Whitney U test.

RESULTS

PTZ thresholds in ST8SiaIV and NCAM knockout mice

Thresholds for myoclonic, clonic, and tonic seizure activity induced by PTZ in ST8SiaIV knockout mice did not differ from those of corresponding wild-type mice (Fig. 1, vehicle data, white columns). PTZ seizure thresholds were in the same range in NCAM knockout mice, wild-type mice as well as mice heterozygous for the genetic deficiency of NCAM (Fig. 2, vehicle data, white columns).

Effect of NBQX in ST8SiaIV knockout mice and wild-type mice

NBQX (80 mg/kg i.p.) induced a significant rise in thresholds for generalized and tonic seizure activity in ST8SiaIV knockout mice (Fig. 1). Drug-induced increases of these thresholds ranged from 37 to 177%. In wild-type mice no significant increases in seizure thresholds were observed in response to NBQX. Differences to the effect of the same dosage of NBQX in NMRI mice in a previous study (Löscher and Hönack, 1994) are likely to be due to genetic