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Differential changes in induced seizures after hippocampal treatment of rats with an antisense oligodeoxynucleotide to the $GABA_A$ receptor $\gamma 2$ subunit

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Abstract

γ-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the brain. Impairment of GABAergic neurotransmission may be involved in the pathogenesis of epileptic phenomena. We have previously characterized biochemical and histological changes following unilateral intrahippocampal infusion of a phosphorothioate antisense oligodeoxynucleotide to the $GABA_A$ receptor $\gamma 2$ subunit in rats in vivo. The aim of the present study was to investigate the behavioral changes of rats following unilateral hippocampal antisense 'knockdown' of the $GABA_A$ receptor $\gamma 2$ subunit. Antisense, but not mismatch control oligodeoxynucleotide treated rats had a significant weight loss (10%) during 6 d of treatment. Antisense treated rats exhibited no changes in spontaneous behavior, including anxiety-like behavior as measured in the social interaction test, compared to mismatch oligodeoxynucleotide treated rats. However, antisense treated rats developed pronounced changes in induced seizure activity. Seizures induced by subcutaneously injected pentylenetetrazol were markedly accentuated in antisense treated rats compared to treatment naive rats, whereas mismatch treated rats showed a lower seizure score than that of naive rats. Antisense treated rats had a significantly elevated threshold for seizures induced by electrical stimulation in the maximal electroshock seizure threshold test. The results suggest that intrahippocampal infusion of antisense oligodeoxynucleotide to the $GABA_A$ receptor $\gamma 2$ subunit leads to specific alterations in the sensitivity to induced seizures. The results are viewed as consequences of selective down-regulation of $GABA_A$ receptors and diminished inhibitory neurotransmission in the hippocampus. © 1997 Elsevier Science B.V.

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1. Introduction

The balance between inhibitory (mainly mediated by γ-aminobutyric acid (GABA)) and excitatory (mainly glutamatergic) neuronal input is crucial for maintaining the normal neuronal cellular functions in the central nervous system (Hablitz, 1984; Johansen and O'Hare, 1989; Thompson, 1993; Gerfin-Moser et al., 1995). Shifting the balance in favor of increased excitatory neurotransmission can elicit epileptiform phenomena (Hablitz, 1984; Thompson, 1993). The involvement of GABA in the regulation of seizure excitability in experimental animals and in humans has been investigated extensively (Meldrum, 1979; Löscher, 1985; Meldrum, 1989; Deyn et al., 1990; Sloviter, 1991; Bekenstein and Lothman, 1993); impairment of GABA-mediated neuronal inhibition can result in seizures, whereas enhancement of GABAergic neurotransmission generally leads to anticonvulsant effects. Diminished GABAergic neuronal inhibition produced in vitro by the addition of GABA antagonists to organotypic hippocampal slice cultures leads to neuronal cell death (Thompson, 1993).

GABA mediates neuronal inhibition mainly via the $GABA_A$ receptor (for review see Macdonald and Olsen, 1994; Sieghart, 1995). Several clinically important compounds, e.g. 1,4-benzodiazepines and barbiturates, exert their actions via binding sites within the $GABA_A$ receptor complex. The $GABA_A$ receptor is part of a multi-subunit

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protein complex assembled from different combinations of polypeptide subunits, of which several families and isotypes have been identified (6 α's, 4 β's, 3 γ's, 1 δ and 2 ρ's) (Sieghart, 1995). Only $GABA_A$ receptor complexes which contain a $\gamma 2$ subunit exhibit classical benzodiazepine pharmacology (Pritchett et al., 1989). The $\gamma 2$ subunit is probably a constituent of the majority of brain $GABA_A$ receptors (Fritschy and Möhler, 1995).

One way of indirectly favoring neuronal excitation could be via a selective down-regulation of $GABA_A$ receptors. Antisense oligodeoxynucleotides can be used for the selective inhibition of the expression of proteins in the brains of experimental animals in vivo (for review see e.g. Wahlestedt, 1994; Heilig and Schlingensiepen, 1996). The mechanism by which an antisense oligonucleotide exerts its action is not fully understood but may be via sequence-specific hybridization to the mRNA encoding the protein of interest. This is thought to lead to either a blockade of the translation of the mRNA or to specific degradation of the mRNA/antisense oligodeoxynucleotide duplex.

We have used an antisense oligodeoxynucleotide complementary to a section of the $GABA_A$ receptor $\gamma 2$ subunit mRNA to selectively inhibit the synthesis of the $\gamma 2$ subunit protein. Biochemical and histological changes following continuous intrahippocampal infusion of the antisense oligodeoxynucleotide to rats have been characterized previously (Karle et al., 1995, 1997a,b). Ex-vivo binding of [^{3}H]flunitrazepam was significantly decreased in antisense treated hippocampi as measured by homogenate binding (43% compared to untreated control) (Karle et al., 1995) and autoradiography (Karle et al., 1997a). Parallel decreases in the binding of ligands to the GABA binding site and the ion channel domain of the $GABA_A$ receptor (Karle et al., 1995) suggested that the blocked expression of the $\gamma 2$ subunit leads to impaired or incomplete assembly of $GABA_A$ receptor complexes meant to contain a $\gamma 2$ subunit. Severe neuronal cell death occurring in the antisense treated hippocampus after about four days of continuous antisense treatment is hypothesized to be a result of a diminished ability of hippocampal neurones to receive GABAergic postsynaptic inhibitory input, due to a decrease in the number of functional $GABA_A$ receptors.

The nucleotide sequence of the antisense oligodeoxynucleotide was essential because benzodiazepine binding was not or only slightly affected by treatment with control sense or mismatch oligodeoxynucleotides. The control oligodeoxynucleotides did not induce histological changes except for a small lesion in the vicinity of the oligodeoxynucleotide infusion site (Karle et al., 1995, 1997b). The sequence specificity of the effects induced by the antisense oligodeoxynucleotide is underscored by the recent observation that an oligodeoxynucleotide identical to the antisense oligodeoxynucleotide except for the sequence of the two central bases, had no effect on benzodiazepine receptor binding (Karle et al., 1997b). The benzodiazepine receptor agonist diazepam, when administered intraperitoneally to rats concurrently with the intrahippocampal antisense treatment, can induce protection against the hippocampal neurodegeneration (Karle et al., 1997b), supporting the hypothesis that the neuronal cell death is a consequence of diminished GABAergic inhibition.

In the present study the behavior of rats treated with unilateral intrahippocampal infusion of antisense oligodeoxynucleotide to the $GABA_A$ receptor $\gamma 2$ subunit was investigated. Rats were examined for adverse behavior upon handling, spontaneous locomotion and social interaction (Sams-Dodd, 1995). Also, rats were examined for induced seizure activity in two well characterized models; the maximal electroshock seizure threshold test is viewed as a model for grand mal tonic seizures (Löscher et al., 1991a); the subcutaneous (s.c.) pentylenetetrazol test is generally regarded as a model for petit mal seizures (Löscher et al., 1991b).

2. Materials and methods

2.1. Animals

Nine week old male Wistar rats (Moellegaard Breeding, Denmark) weighing 250–290 g were used for all experiments. Rats were housed in individual cages at room temperature under a natural day–night cycle, with access to tap water and commercial food pellets ad libitum. Rats were kept in the laboratory for approximately one week before the time of surgery.

2.2. Oligodeoxynucleotides

Eighteen-mer fully phosphorothioate-modified oligodeoxynucleotides (DNA Technology, Aarhus) were used. The antisense sequence is (5′-TAT–TTG–GCG–AAC–TCA–TCG-3′) (Karle and Nielsen, 1995), complementary to the putative translation initiation codon of the $GABA_A$ receptor $\gamma 2$ subunit mRNA (Shivers et al., 1989). A mismatch oligodeoxynucleotide, in which the sequence of the four central bases was interchanged (5′-TAT–TTG–GAA–GCC–TCA–TCG-3′) was used as control. Oligodeoxynucleotides were purified by reverse-phase high pressure liquid chromatography (HPLC) and dissolved in sterile H_2O (1.7 μg/μl) for intrahippocampal infusion.

2.3. Intrahippocampal oligodeoxynucleotide infusion

Antisense or mismatch oligodeoxynucleotide or sterile H_2O was infused continuously into the right hippocampus of rats as previously described (Karle et al., 1995). Briefly, rats were anaesthetized (sodium pentobarbital, 50 mg/kg body weight i.p.) and a cannula was stereotactically implanted centrally in the hippocampus ($A = 3.0$ mm; $L = +4.3$ mm from aural line; depth: 5.0 mm inferior to skull). The cannula was connected to an Alzet osmotic minipump (Alza, Palo Alto, CA) via a polyethylene catheter.

Groups of rats received either the antisense oligodeoxynucleotide (1.7 μg/μl; 0.5 μl infusion/h), ($n = 7$ in each experiment) or the mismatch control oligodeoxynucleotide (1.7 μg/μl; 0.5 μl infusion/h), ($n = 7$). As additional controls either rats infused with vehicle (sterile H_2O), ($n = 7$) or treatment-naive rats ($n = 7$) were included. The additional control group used in the individual experiments is indicated in Section 3. Rats were used for one experiment only, except in the tests for spontaneous locomotion and social interaction, where the same rats were tested in both paradigms after five and six days of continuous oligodeoxynucleotide infusion, respectively. Rats were tested in the seizure models after continuous treatment for five or six days.

2.4. Rating for adverse behavior upon handling

A rating scale for adverse behavior was constructed. All animals were handled by a blinded observer after one, four and five days of continuous intrahippocampal infusion (infusion was initiated on day 0). Adverse behavior was scored according to the following four-step rating scale with treatment naive rats as reference: 0: less adverse than naive rats; 1: equal to naive rats; 2: moderately adverse, e.g. resisting handling, turning head in attempt to bite, stiffening of back when held, but fairly easy to calm down; 3: severely adverse reaction, i.e. screaming, fighting to become loose, continuously uneasy when held. Median scores and 25% and 75% quartiles of ratings of the tested groups (antisense, mismatch and vehicle treated rats) were calculated and compared by the Mann–Whitney *U*-test.

2.5. Spontaneous locomotion

Rats were tested individually for spontaneous locomotion in a test cage equipped with light sources and photocells (Arnt, 1995). Recording of a motility count required the consecutive interruption of adjacent light beams; motility was evaluated for a period of two hours for each animal. Mean motility counts ($\pm$S.E.M.) were compared by one way analysis of variance (ANOVA).

2.6. Social interaction test

The social interaction test was originally designed by File (1980) and modified by Sams-Dodd (1995, 1996). Briefly, the test was carried out in an open arena (*L*, *W*, *H*: $150 \times 100 \times 40$ cm) equipped with overhead videocameras. Antisense or mismatch oligodeoxynucleotide treated or naive rats were paired with untreated rats from a separate group. The untreated partner rats were dyed with black hair color to allow computer image recognition from videotapes. One rat from the experimental group and one from the untreated partner group were placed simultaneously into the unfamiliar arena (30–40 cm apart) one hour after the beginning of the animals' night cycle. The behavior of rats was recorded by a video camera for 10 min and analyzed by the EthoVision® program (Noldus Information Technologies). The analysis resulted in a track record for each rat that contained a complete record of the rat's movement pattern in the arena during the observation period. The track records were analyzed for the following parameters: the total *distance travelled* (cm) during an observation period. Percentage of the 10 min observation *time spent in the central zone* (central 33%) of the arena. Duration of active *social interaction*, defined as the duration of periods (s) in which the distance between the rats was less than 20 cm and in which the rats were active. Results were compared by one way ANOVA.

2.7. Maximal electroshock seizure threshold

The threshold for maximal electroshock seizures (induction of tonic hind limb extension) was determined by means of the 'up-and-down' method (Löscher et al., 1991a) using transcorneal electrodes. Briefly, rats were fixed and one drop of saline (0.9%) was applied to the cornea on both sides. A constant current (50 Hz, 0.5 s) was applied by means of a stimulator (Puls-stimulator, DCM Electronics, Copenhagen). An initial stimulus intensity was chosen based on the expected threshold current inducing tonic hind limb extension in 50% of the rats (CC_{50}). If the animal exhibited tonic hind limb extension, the intensity was lowered by 5 mA for the subsequent animal to be tested. If no hind limb extension was observed, the stimulus intensity was raised by 5 mA for the next animal. Each animal was tested only once. The CC_{50} was calculated by the method of Kimball et al. (1957). CC_{50} values for the different groups of rats were compared by the *t*-test.

2.8. Subcutaneous pentylenetetrazol seizure test

Pentylenetetrazol (Unikem, Copenhagen) was dissolved in saline (0.9%) and injected s.c. A dose of 70 mg/kg (in a volume of 5 ml/kg) was chosen because this dose induces clonic seizures in the majority of rats. Rats were observed by an observer unaware of the treatment sequence during 30 min following the pentylenetetrazol injection. The appearance of the following types of seizures (Löscher et al., 1991b) was noted for each animal: 0: no seizure; 1: generalized myoclonic twitches; 2: generalized clonic seizure (without loss of righting reflexes); 3: generalized clonic seizure (with loss of righting reflexes); 4: loss of righting with tonic forelimb seizure; 5: loss of righting with tonic fore- and hindlimb seizure. We included a score of 6 indicating recurrent seizures. The median seizure score for each group with 25% and 75% quartiles was calculated. The maximal seizure scores were compared by means of the Mann–Whitney *U*-test.

3. Results

3.1. General observations

One rat died shortly after the implantation of the infusion cannula and osmotic minipump filled with mismatch oligodeoxynucleotide; the death is most likely not attributable to infusion of the mismatch oligodeoxynucleotide. Otherwise there was no lethality. Within the study period none of the rats exhibited spontaneous behavioral seizures or gross abnormal behavior.

Antisense treated rats had a loss of body weight from 265 ± 11 g (mean $\pm$ S.D.) (surgery day) to 238 ± 21 g (day 6 post surgery), ($P < 0.03$; Mann–Whitney *U*-test). None of the control rats lost weight during six days after surgery (mismatch: 265 ± 8 g at surgery; 271 ± 10 g on day 6 post surgery).

3.2. Rating for adverse behavior upon handling

There were no differences among the groups in scores obtained with the rating scale for adverse behavior. On day 6 post surgery the scores were (median; (25% and 75% quartiles)): Antisense: 2 (1–2); Mismatch: 1 (1–2); Vehicle: 1 (1–2).

3.3. Spontaneous locomotion

There were no differences in the motility counts of the three tested groups of rats (antisense, mismatch and naive) (Fig. 1).

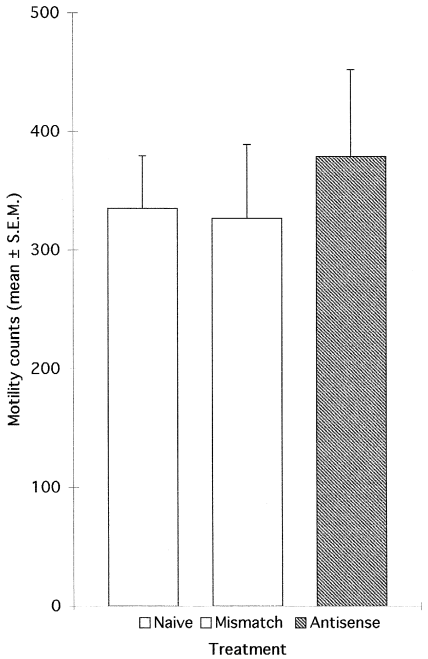

Fig. 1. Results of spontaneous locomotion test. Data are expressed as mean motility counts per two hours ($\pm$S.E.M.) of rats ($n = 7$ in all groups).

3.4. Social interaction test

In the social interaction test the behavior of antisense rats did not differ from that of mismatch rats in any of the three measured parameters (Fig. 2). Both oligodeoxynucleotide treated groups (antisense and mismatch) showed a small decrease ($P < 0.05$) in *travelled distance* compared to naive animals (Fig. 2A).

3.5. Maximal electroshock seizure threshold

Two independent experiments were carried out. Pooled results of the two maximal electroshock seizure threshold experiments are shown in Fig. 3. In the first experiment three groups of rats were tested: antisense, mismatch and naive ($n = 7$ in all groups in both experiments). In naive rats the CC_{50} value ($\pm$S.E.M.) was 55 ± 3.7 mA, indicating that 50% of the rats exhibit tonic hind limb extension at a stimulus intensity of 55 mA. The CC_{50} value for mismatch rats (51 ± 2.8 mA) was not significantly different from that of naive rats. Antisense rats showed a threshold for the development of tonic hindlimb extension above 65 mA. A second experiment including antisense, mismatch and vehicle treated rats was carried out to precisely determine the CC_{50} value for rats treated with antisense oligodeoxynucleotide. Based on the findings of the first experiment an initial stimulus intensity of 70 mA was chosen for the antisense rats. This experiment revealed a CC_{50} of antisense rats of 79.2 ± 2.8 mA (Fig. 3). The value was significantly different from those of control rats ($P < 0.05$): mismatch rats (47.5 ± 6.4 mA) and vehicle rats (62.5 ± 6.4 mA). The CC_{50} values of the two control groups did not differ significantly from each other.

3.6. Subcutaneous pentylenetetrazol seizure test

Treatment naive rats had a median seizure score of 4 (25% and 75% quartiles: 4–4) (Table 1). Mismatch rats showed a lower seizure score of 3 (1–3.75) ($P < 0.05$), reflecting that these rats did not lose the righting reflexes during the pentylenetetrazol induced seizure attack. Antisense rats exhibited a significantly higher seizure score (5, 5–6) ($P < 0.05$), indicating severe and often recurrent convulsions. Three of the antisense rats developed recurrent seizures with extensive tonic–clonic convulsions (score 6). These three rats were killed prematurely in order to prevent excessive suffering. In a second experiment including vehicle treated rats instead of naive rats, a notable increase of seizure score of antisense treated rats (6, 2.25–6) was again observed (Table 1). However, in this experiment the difference between the scores of antisense rats and control rats did not reach statistical significance. In the second experiment, four antisense rats who devel-

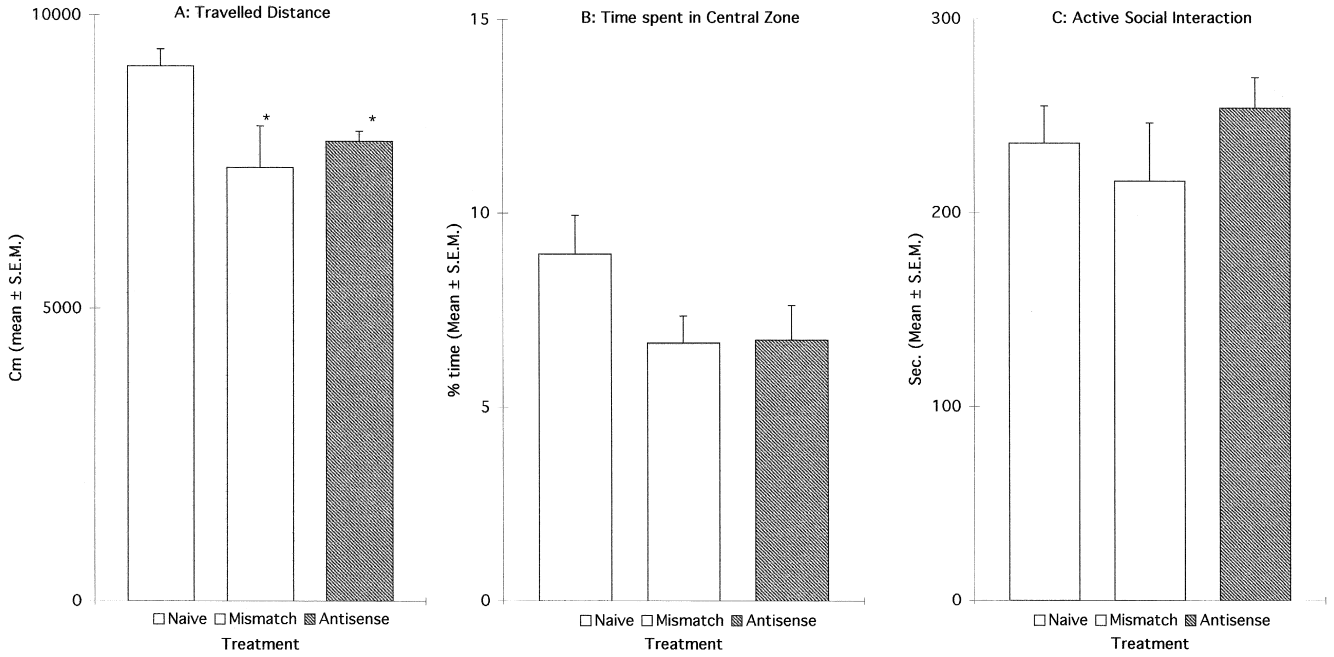

Fig. 2. Results of the social interaction test. Data are expressed as mean ± S.E.M. ($n = 7$ in all groups). (A) Distance travelled (cm). (B) % time spent in central zone of test arena (s). (C) Duration of active social interaction (s). $^{*}P < 0.05$ versus naive (one-way ANOVA).

oped degree 6 convulsions were killed prematurely. The overall result was that recurrent tonic convulsions were induced in antisense animals by a dose of pentylenetetrazol generating clonic convulsions in control rats.

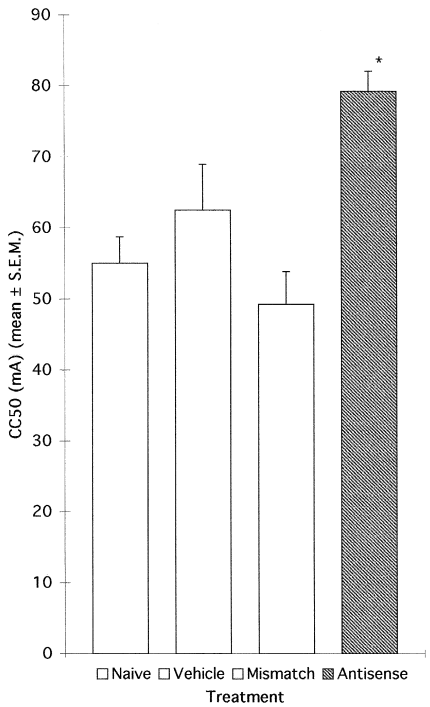

Fig. 3. Results of maximal electroshock seizure threshold experiments. CC_{50} values are expressed as mean ± S.E.M. Note that the CC_{50} for the mismatch oligodeoxynucleotide treated group of rats (MM) is the mean of values from two experiments. $^{*}P < 0.05$ versus naive, vehicle and mismatch oligodeoxynucleotide treated rats (*t*-test).

4. Discussion

The present report describes the results of behavioral and seizure tests of rats treated with continuous unilateral intrahippocampal infusion of an antisense oligodeoxynucleotide to the $GABA_A$ receptor $\gamma 2$ subunit. The antisense oligodeoxynucleotide treated rats exhibited no spontaneous seizures or gross abnormal behavior during the treatment period (up to six days). Also, antisense treated rats showed no behavioral differences compared to control rats when handled, in spontaneous locomotion, or in the social interaction test. However, the antisense treated rats showed a significant weight loss (10%). Furthermore, the antisense treatment led to pronounced effects on induced seizures as observed in the maximal electroshock seizure threshold test and the s.c. pentylenetetrazol test. Antisense rats had a markedly elevated threshold for the induction of tonic hindlimb extension by means of electrostimulation, whereas injection of pentylenetetrazol resulted in severe and often recurrent tonic convulsions at a dose of pentylenetetrazol which induced clonic convulsions in control rats.

We believe that the primary event causing the changes in seizure activity is the selective inhibition of the expression of the $GABA_A$ receptor $\gamma 2$ subunit. It is shown that the nucleotide sequence of the antisense oligodeoxynucleotide is essential for the induction of the changes in seizure activity. We have used a mismatch control oligodeoxynucleotide with a nucleotide sequence closely resembling that of the antisense oligodeoxynucleotide. The seizure activity of rats treated with the mismatch oligodeoxynucleotide did not differ from that of naive or vehicle treated rats in the maximal electroshock seizure

threshold test. In the s.c. pentylenetetrazol test, mismatch treated rats had a lower seizure score than naive rats (Table 1). This finding is presently unexplained.

The reported behavioral effects are accompanying hippocampal changes which have been characterized previously in our laboratory and which have been shown to be nucleotide sequence specific (see Section 1).

When handled, in spontaneous locomotion and in social interaction, the behavior of antisense rats was not distinguishable from that of control rats. In the social interaction test, both antisense and mismatch rats travelled for a shorter distance than naive rats. The decrease in travelled distance may be attributable to the surgical procedures, since it involved both the groups that underwent surgery. The differences between the spontaneous locomotion and the travelled distance in the social interaction test may reflect the differences between the designs of the two tests. Rats are tested for spontaneous locomotion in a bright environment during daytime. This test was designed to monitor hyperactivity; normal rats quickly fall asleep in the test cage. The social interaction test, which is carried out in a dark environment during the night, was designed to measure decreases in activity.

No differences were observed in the time spent in the central zone of the social interaction arena, indicating that antisense rats showed the same level of anxiety-like behavior as control rats. Also, there were no differences between the antisense and control groups in the duration of active social interaction with an untreated partner rat. Taken together, antisense rats appeared normal except for the decreased level of activity at night and the weight loss. The loss of body weight suggests that the rats become anorectic by the antisense treatment; it cannot be excluded that this factor contributes to the effects seen in the seizure tests. Nevertheless, we do not believe that the differences in convulsive thresholds can be explained by a general derangement of the animals. The apparently selective effect on seizure behavior in antisense rats is therefore probably not merely a result of severe brain damage. Within the period studied in the present experiments the histological changes and the changes in benzodiazepine receptor binding are essentially restricted to the antisense treated hippocampus (Karle et al., 1997a).

It is interesting that the antisense treated rats did not develop spontaneous behavioral seizures. Generalized seizures were not observed in rats left for up to six weeks after discontinuation of antisense oligodeoxynucleotide infusion (J. Karle, unpublished observation). It is possible, perhaps likely, that the rats develop electroencephalographically detectable seizures. Intrahippocampal injection of compounds which impair GABAergic neurotransmission, e.g. bicuculline, picrotoxin or isoniazid, produces limbic seizure activity (Meldrum, 1989). Following unilateral intrahippocampal injection of the excitotoxin kainic acid, rats develop limbic seizures, characterized by e.g. grooming, locomotor activity, wet-dog shakes and oral stereotypies (French et al., 1982); at higher doses generalized seizures have been reported (Schwarcz et al., 1978). After unilateral electrical stimulation of the perforant path (Sloviter, 1987, 1991), rats did not develop motor seizures. Metabolic changes or decreased respiration associated with motor seizure activity are not the underlying causes of the antisense induced hippocampal neurodegeneration (Karle et al., 1995, 1997b), since the antisense rats showed no motor seizures.

It has yet to be determined whether the observed changes in seizure propensity can be attributed to the $GABA_A$ receptor $\gamma 2$ subunit knockdown per se, or whether the changes are a result of the hippocampal neuronal cell death or compensatory mechanisms. Neuronal cell death has been observed after about 4 d of antisense infusion (Karle et al., 1997b); the seizure tests were carried out after 5 or 6 d of infusion. The observed changes in induced seizure activity may not be explained solely in terms of changes in GABAergic neurotransmission, since the primary effect apparently elicits a cascade of effects ultimately resulting in neurodegeneration. Recently, it was shown by Zhao et al. (1996) that rats treated with intracerebroventricular (i.c.v.) injections of a 17-mer $GABA_A$ receptor $\gamma 2$ subunit antisense oligodeoxynucleotide with a sequence closely resembling the one used in our laboratory had an increased theshold for the induction of convulsions by a benzodiazepine receptor inverse agonist. The threshold for seizures induced by picrotoxin was unchanged.

S.c. pentylenetetrazol injection induced convulsions of increased severity in the antisense rats. This may not be surprising, since e.g. rats treated systemically (Sperk, 1994) or intrastriatally (Pisa et al., 1980) with kainic acid are more susceptible than normal rats to convulsions induced by pentylenetetrazol. It is conceivable that a hippocampal lesion which is the result of diminished inhibitory neurotransmission could lead to a potentiation of pentylenetetrazol induced seizures.

The elevation of the tonic convulsion threshold of antisense rats in the maximal electroshock seizure threshold test is consistent with an anticonvulsant effect of the antisense treatment against electrically induced tonic seizures. This effect is apparently opposite to the pentylenetetrazol induced changes in seizure behavior. It is likely that different mechanisms involving separate neuronal circuits are responsible for the development of electrically and chemically induced seizures (Mirski et al., 1986; Miller et al., 1987; Löscher et al., 1991b). For example, bilateral intratectal injections of bicuculline methiodide, a $GABA_A$ receptor antagonist, resulted in an anticonvulsant effect against maximal electroshock seizure convulsions and a worsening of i.v. pentylenetetrazol induced convulsions in rats (Weng and Rosenberg, 1992). The effects of the blocked expression of the $GABA_A$ receptor $\gamma 2$ subunit in a model for tonic seizure threshold versus a model for clonic seizures, are not immediately analogous to other conditions following unilateral hip-

pocampal changes. In our condition the elevated CC_{50} of antisense rats may be a result of compensatory effects that have taken place in the antisense treated brains, and which may act to prevent the spread of seizure activity. The increased severity of convulsions induced by pentylenetetrazol may be viewed as a result of an increased propensity for excitation in the lesioned hippocampus. In other words, the accentuation of pentylenetetrazol induced convulsions may be attributed to the primary effect in the antisense treated hippocampus. We speculate that a decrease of GABAergic tonus in the antisense treated hippocampus can explain the worsening of pentylenetetrazol convulsions. A generalized increase of GABAergic tonus as a compensation for a regional down-regulation of $GABA_A$ receptors could be the cause of the elevated seizure threshold found in the maximal electroshock seizure threshold test.

Several anti-epileptic compounds show anticonvulsant properties in both the applied seizure models. Vigabatrin and progabide, enhancers of GABAergic neurotransmission, have been reported to be ineffective against s.c. pentylenetetrazol induced clonic seizures (Löscher, 1985). At high doses, phenytoin, primidone and carbamazepine have in some studies, but not in others, been found ineffective against pentylenetetrazol induced clonic seizures (Frey, 1985; Jones and Wimbish, 1985; Schmutz, 1985; Löscher et al., 1991b; Palmer et al., 1992). All the mentioned compounds are effective against electrically induced tonic convulsions.

In conclusion, discrete behavioral changes were obtained after the unilateral hippocampal antisense inhibition of the synthesis of the $GABA_A$ receptor $\gamma 2$ subunit in rats. Antisense treated rats showed no changes in spontaneous behavior, but exhibited significant changes in induced seizure behavior.

Future studies, including electroencephalographic investigations, are expected to contribute to an increased understanding of the changes induced by the $GABA_A$ receptor $\gamma 2$ subunit antisense treatment. This animal model may be of relevance for the study of mechanisms of neuronal cell death and of epileptic phenomena.

Furthermore, antisense knockdown of other $GABA_A$ receptor subunits may lead to increased knowledge of the physiological and pharmacological properties of different subunits of the receptor complex.

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References

Arnt, J., 1995. Differential effects of classical and newer antipsychotics on the hypermobility induced by two dose levels of D-amphetamine. Eur. J. Pharmacol. 283, 55–62.

Bekenstein, J.W., Lothman, E.W., 1993. Dormancy of inhibitory interneurons in a model of temporal lobe epilepsy. Science 259, 97–100.

Deyn, P.P.D., Marescau, B., Macdonald, R.L., 1990. Epilepsy and the GABA-hypothesis, a brief review and some examples. Acta Neurol. Belg. 90, 65–81.

File, S.E., 1980. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J. Neurosci. Methods 2, 219–238.

French, E.D., Aldinio, C., Schwarcz, R., 1982. Intrahippocampal kainic acid, seizures and local neuronal degeneration: Relationships assessed in unanesthetized rats. Neuroscience 7, 2525–2536.

Frey, H.-H., 1985. Primidone. In: Frey, H.-H., Janz, D. (Eds.), Antiepileptic Drugs. Handbook of Experimental Pharmacology. Springer, Berlin, vol. 74, pp. 449–477.

Fritschy, J.-M., Möhler, H., 1995. $GABA_A$ receptor heterogeneity in the adult rat brain: Differential regional and cellular distribution of seven major subunits. J. Comp. Neurol. 359, 154–194.

Gerfin-Moser, A., Grogg, F., Rietschin, L., Thompson, S.M., Streit, P., 1995. Alterations in glutamate but not $GABA_A$ receptor subunit expression as a consequence of epileptiform activity in vitro. Neuroscience 67, 849–865.

Hablitz, J.J., 1984. Picrotoxin-induced epileptiform activity in hippocampus: Role of endogenous versus synaptic factors. J. Neurophysiol. 51, 1011–1027.

Heilig, M., Schlingensiepen, K.-H., 1996. Antisense oligodeoxynucleotides as novel neuropharmacological tools for selective expression blockade in the brain. In: Genetic Manipulation of the Nervous System. Academic Press, London, pp. 249–268.

Johansen, F.F., O'Hare, M.M.T., 1989. Loss of somatal neuropeptide Y immunoreactivity in the rat hippocampus following transient cerebral ischemia. J. Neurosurg. Anesthesiol. 1, 339–345.

Jones, G.L., Wimbish, G.H., 1985. Hydantoins. In: Frey, H.-H., Janz, D. (Eds.), Antiepileptic Drugs. Handbook of Experimental Pharmacology. Springer, Berlin, vol. 74, pp. 351–419.

Karle, J., Nielsen, M., 1995. Modest reduction of benzodiazepine binding in rat brain in vivo induced by antisense oligonucleotide to $GABA_A$ receptor $\gamma 2$ subunit subtype. Eur. J. Pharmacol. Mol. Pharmacol. 291, 439–441.

Karle, J., Witt, M.-R., Nielsen, M., 1995. Antisense oligonucleotide to $GABA_A$ receptor $\gamma 2$ subunit induces loss of neurones in rat hippocampus. Neurosci. Lett. 202, 97–100.

Karle, J., Witt, M.-R., Nielsen, M., 1997a. The use of in vivo antisense oligonucleotide technology for the investigation of brain $GABA_A$ receptors. Neurochem. Int. 31, 437–446.

Karle, J., Witt, M.R., Nielsen, M., 1997b. Diazepam protects against rat hippocampal neuronal cell death induced by antisense oligodeoxynucleotide to $GABA_A$ receptor $\gamma 2$ subunit. Brain Res. 765, 21–29.

Kimball, A.W., Burnett, W.T., Doherty, D.G., 1957. Chemical protection against ionizing radiation. I. Sampling methods for screening compounds in radiation protection studies with mice. Radiat. Res. 7, 1–12.

Löscher, W., 1985. GABAmimetics in animal models of seizure states. In: Bartholini, G. et al. (Eds.), L. E. R. S. Raven Press, New York, vol. 3, pp. 109–119.

Löscher, W., Fassbender, C.P., Nolting, B., 1991a. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models. Epilepsy Res. 8, 79–94.

Löscher, W., Hönack, D., Fassbender, C.P., Nolting, B., 1991b. The role of technical, biological, and pharmacological factors in the laboratory

evaluation of anticonvulsant drugs. III. Pentylenetetrazole seizure models. Epilepsy Res. 8, 171–189.

Macdonald, R.L., Olsen, R.W., 1994. $GABA_A$ receptor channels. Annu. Rev. Neurosci. 17, 569–602.

Meldrum, B.S., 1979. Convulsant drugs, anticonvulsants and GABA-mediated neuronal inhibition. In: Krogsgaard-Larsen, P., Scheel-Krüger, J., Kofoed, H. (Eds.), GABA-Neurotransmitters. Munksgaard, Copenhagen, pp. 390–405.

Meldrum, B.S., 1989. GABAergic mechanisms in the pathogenesis and treatment of epilepsy. Br. J. Clin. Pharmacol. 27, 3S–11S.

Miller, J.W., McKeon, A.C., Ferrendelli, J.A., 1987. Functional anatomy of pentylenetetrazol and electroshock seizures in the rat brainstem. Ann. Neurol. 22, 615–621.

Mirski, M.A., McKeon, A.C., Ferrendelli, J.A., 1986. Anterior thalamus and substantia nigra: Two distinct structures mediating experimental generalized seizures. Brain Res. 397, 380–388.

Palmer, G.C., Harris, E.W., Ray, R., Stagnitto, M.L., Schmiesing, R.J., 1992. Classification of compounds for prevention of NMDLA-induced seizures/mortality, or maximal electroshock and pentylenetetrazol seizures in mice and antagonism of MK801 binding in vitro. Arch. Int. Pharmacodyn. 317, 16–34.

Pisa, M., Sanberg, P.R., Corcoran, M.E., Fibiger, H.C., 1980. Spontaneously recurrent seizures after intracerebral injections of kainic acid in rat: A possible model of human temporal lobe epilepsy. Brain Res. 200, 481–487.

Pritchett, D.B., Sontheimer, H., Shivers, B.D., Ymer, S., Kettenmann, H., Schofield, P.R., Seeburg, P.H., 1989. Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. Nature 338, 582–585.

Sams-Dodd, F., 1995. Automation of the social interaction test by a video-tracking system: Behavioural effects of repeated phencyclidine treatment. J. Neurosci. Methods 59, 157–167.

Sams-Dodd, F., 1996. Phencyclidine-induced stereotyped behaviour and social isolation in rats: A possible animal model of schizophrenia. Behav. Pharmacol. 7, 3–23.

Schmutz, M., 1985. Carbamazepine. In: Frey, H.-H., Janz, D. (Eds.), Antiepileptic drugs. Handbook of Experimental Pharmacology. Springer, Berlin, vol. 74, pp. 479–506.

Schwarcz, R., Zaczek, R., Coyle, J.T., 1978. Microinjection of kainic acid into the rat hippocampus. Eur. J. Pharmacol. 50, 209–220.

Shivers, B.D., Killisch, I., Sprengel, R., Sontheimer, H., Köhler, M., Schofield, P.R., Seeburg, P.H., 1989. Two novel $GABA_A$ receptor subunits exist in distinct neuronal subpopulations. Neuron 3, 327–337.

Sieghart, W., 1995. Structure and pharmacology of γ-aminobutyric $acid_A$ receptor subtypes. Pharmacol. Rev. 47, 181–234.

Sloviter, R.S., 1987. Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy. Science 235, 73–76.

Sloviter, R.S., 1991. Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: The 'Dormant Basket Cell' hypothesis and its possible relevance to temporal lobe epilepsy. Hippocampus 1, 41–66.

Sperk, G., 1994. Kainic acid seizures in the rat. Prog. Neurobiol. 42, 1–32.

Thompson, S.M., 1993. Consequences of epileptic activity in vitro. Brain Pathol. 3, 413–419.

Wahlestedt, C., 1994. Antisense oligonucleotide strategies in neuropharmacology. Trends Pharmacol. Sci. 15, 42–46.

Weng, X., Rosenberg, H.C., 1992. Infusion of bicuculline methiodide into the tectum: Model specificity of pro- and anticonvulsant actions. Epilepsy Res. 12, 1–8.

Zhao, T., Rosenberg, H.C., Chiu, T.H., 1996. Treatment with an antisense oligodeoxynucleotide to the $GABA_A$ receptor $\gamma 2$ subunit increases convulsive threshold for β-CCM, a benzodiazepine 'inverse agonist', in rats. Eur. J. Pharmacol. 306, 61–66.