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# Functional characterization and expression of thalamic GABA<sub>B</sub> receptors in a rodent model of Parkinson's disease

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## Abstract

Increased GABAergic neurotransmission of the basal ganglia output nuclei projecting to the motor thalamus is thought to contribute to the pathophysiology of Parkinson's disease. We investigated the functional role of thalamic GABA<sub>B</sub> receptors in a rodent model of Parkinson's disease. First, we examined the effects of blockade of GABA<sub>B</sub> receptors in the ventromedial thalamic nucleus of rats with a unilateral 6-OHDA lesion of the substantia nigra on locomotor activity. In addition we studied the expression of GABA<sub>B</sub> receptor mRNA in the basal ganglia and thalamus using *in situ* hybridisation. Unilateral microinjections of the GABA<sub>B</sub> receptor antagonist 2-hydroxysaclofen into the ventromedial thalamic nucleus ipsilateral to the nigrostriatal lesion induced contralateral rotations in a dose-dependent manner. However, microinjection of antagonists with higher affinity for the GABA<sub>B</sub> receptor SCH 50911, CGP 56433 and CGP 55845 did not result in rotational behaviour, but did induce convulsions at higher doses. GABA<sub>B</sub> receptor mRNA expression was found throughout the basal ganglia and thalamus, including the ventromedial thalamic nucleus. No statistically significant differences in GABA<sub>B</sub> mRNA expression were observed in the ventromedial thalamic nucleus following a unilateral 6-OHDA lesion of the substantia nigra. These results make it improbable that thalamocortical GABA<sub>B</sub> receptors play an important role in the pathophysiology of parkinsonism. Therefore, GABA<sub>B</sub> receptors do not appear to be a promising target for novel antiparkinsonian drugs. © 1999 Elsevier Science Ltd. All rights reserved.

**Keywords:** Parkinson's disease; GABA<sub>B</sub> receptor; Thalamus; Motor behaviour; *In situ* hybridisation

## 1. Introduction

The understanding of the pathophysiology of Parkinson's disease (PD) has improved considerably in the past decade. The progressive degeneration of dopaminergic neurones of the substantia nigra pars compacta, resulting in depletion of dopamine within the basal ganglia leads to overactivity of the lateral globus pallidum (LGP) and disinhibition of the subthalamic nucleus (STN). The glutamatergic fibres arising from the STN in turn cause excessive excitation of the basal ganglia output nuclei, substantia nigra reticulata (SNr) and the medial globus pallidum (MGP) (Albin et al.,

1989; DeLong, 1990; Klockgether and Turski, 1993; Chesselet and Delfs, 1996). Electrophysiological studies in animal models of PD demonstrated higher tonic firing rate at rest, increased probability of bursting activity and abnormal coupling of neighboring neurones (Filion and Tremblay, 1991; Bergmann et al., 1994; Wichman and DeLong, 1996). The output neurones of the SNr and MGP are GABAergic and in rodents project to the ventromedial thalamic nucleus (VM), which in turn projects to the cortex (Herkenham, 1979; Kilpatrick et al., 1980; MacLeod et al., 1980). Increased basal ganglia output in PD therefore should result in increased GABAergic inhibition of the motor-thalamus and decreased activation of the motor cortex (Jenkins et al., 1992; Wichman and DeLong, 1996). In spite of the presumed critical role in the pathophysiology of PD, little attention has been paid to GABAergic transmission in the thalamus. We and others have

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shown previously that baclofen, an agonist at the recently cloned GABA<sub>B</sub> receptor, induces akinesia when applied locally into the VM suggesting that increased thalamic GABA<sub>B</sub> receptor-mediated inhibition plays a role in parkinsonism (DiChiara et al., 1979; Klockgether et al., 1985, 1986; Wüllner et al., 1987). The current model of basal ganglia functioning predicts that lesions of motorparts of the thalamus result in akinesia (Albin et al., 1989; Lang and Lozano, 1998). However, lesions of motor parts of the thalamus in humans do not induce parkinsonism and thalamotomy in PD patients improves the symptoms of PD instead of worsening them, suggesting that not a simple loss of function but rather changes in neural firing patterns underlie the symptoms of Parkinson's disease (Marsden and Obeso, 1994). This hypothesis is particularly attractive since tonic activation of thalamic GABA<sub>B</sub> receptors in vitro results in oscillatory neuronal activity (Crunelli and Leresche, 1991; Williams et al., 1995). Indeed, electrophysiological recordings in parkinsonian monkeys revealed that the increased GABAergic inhibition of the thalamus in these monkeys was associated with abnormal oscillatory behaviour of thalamic neurones (Vitek et al., 1994). To test the hypothesis that disturbances of basal ganglia activity leading to parkinsonism are mediated by abnormally increased thalamic GABA<sub>B</sub> inhibition we investigated whether blockade of GABA<sub>B</sub> receptors in the VM of 6-OHDA lesioned rats has antiparkinsonian effects. In this animal model antiparkinsonian drugs induce contralateral rotational behaviour. In addition, we studied whether lesions of the nigrostriatal dopaminergic system alter the expression of GABA<sub>B</sub> receptors in the thalamus.

## 2. Methods

### 2.1. Subjects

Male Wistar rats (Charles River, Sulzfeld, Germany) were housed in groups of three under a 12 h light–dark cycle with lights on at 06:30 h. Water and food were available ad libitum. All procedures were conducted in accordance with the animal welfare guides and laws of the Federal Republic of Germany.

### 2.2. Surgery

Rats (200–220 g) were anaesthetised with methohexital (50 mg/kg i.p., Lilly) and placed in a stereotaxic apparatus (Stoelting). A total of 16 µg 6-hydroxydopamine (6-OHDA, Sigma) dissolved in 4 µl 0.9% sterile saline containing 0.02% ascorbic acid was injected into the left SNc. The stereotaxic coordinates were: 5.5 mm posterior to Bregma, 2.2 mm left from midline and 8.0 mm ventral to the surface of the skull (Paxinos and

Watson, 1998). Thirty minutes before stereotaxic injection of 6-OHDA into the SNc, rats received an injection of pargyline (50 mg/kg s.c., Sigma) to increase 6-OHDA toxicity. One week after the lesion rotational behaviour was quantified following a single injection of apomorphine (0.1 mg/kg s.c., Sandoz). Rats rotating more than 100 times in thirty minutes were used for subsequent studies. Earlier studies in our laboratory demonstrated that this correlates with the loss of more than 95% tyrosine hydroxylase positive neurones in the SNc (Loschmann et al., 1997). Two months after the lesion, a 10 mm long guide cannula (26 gauge stainless steel) was implanted in the skull under methohexital anaesthesia. The cannula was secured to the skull with dental acrylate and stainless screws, and protected from obstruction by a 33 gauge obturator. The coordinates of the guide cannula were 2.9 mm posterior to Bregma, 1.4 mm left from midline and 1.0 mm ventral to the surface of the skull. Rats were allowed to recover one week before the first intrathalamic microinjection.

### 2.3. Drugs

The following drugs were used; 2-hydroxysaclofen ((*RS*)-3-amino-2-(4-chlorophenyl)-2-hydroxy propyl -sulphonic acid, RBI), CGP 56433 ([3-[[1-(*S*)-[3-Cyclohexylmethyl-hydroxyphosphinyl]-2-(*S*)-hydroxy-propyl]amino]ethyl]-benzoic acid) and CGP 55845 ([3-[1-(*S*)-(3,4-Dichlorophenyl) amino-2-(*S*)-hydroxy-propyl]-phenylmethyl-phosphinic acid hydroxychloride) were gifts of Dr Froestl (Novartis Pharma AG, Basel, Switzerland) and SCH 50911 ((+)-(2*S*)-5,5-Dimethyl-2-morpholineacetic acid) was donated by Dr Kreutner (Schering-Plough Research Institute, Kenilworth, USA). All drugs were dissolved in sterile saline pH 7.0 (DeltaPharma, Pfulling, Germany). All dosages refer to the free drug form.

### 2.4. Rotational behaviour

Ipsiversive and contraversive rotations were registered by means of an automatic device consisting of 6 perspex bowls (40 cm in diameter) and electro-mechanical transducer systems that registered a rotation each time the animal rotated through 360°. Rotations were accumulated in 5 min intervals and recorded for 60 min. All experiments were performed between 09:00 and 14:00 h.

For microinjection of the drugs, a 17.5 mm long 33 gauge cannula was carefully inserted in the guide cannula. A total of 0.5 µl was injected over a 5 min period using a Hamilton syringe. The cannula was left in place for one minute afterwards. The injection procedure was performed in the same cage where rotational behaviour was measured. Rats were placed in this cage 1 h before the microinfusion started. Measurement of rotational

behaviour started directly after removing the inserting cannula. Rats received no more than two microinjections, with a wash-out period of at least one week between each experiment.

For verification of the drug injection site, rats were decapitated one day after the last microinfusion. Brains were removed quickly and frozen in isopentane at  $-40^{\circ}\text{C}$ . Sections ( $20\ \mu\text{m}$ ) through the thalamus were stained with thionine (Sigma) and analysed under a light microscope.

### 2.5. *In situ* hybridization

For *in situ* hybridisation, a group of unilateral nigrostriatal lesioned rats with no other intracerebral injections, were decapitated two months after the lesion. Brains were removed and frozen in isopentane at  $-40^{\circ}\text{C}$ . Twenty micrometre sections were cut on a cryostat microtome and stored at  $-80^{\circ}\text{C}$  until further processing. Oligodeoxynucleotide probes (40 basepairs) were constructed according to the published DNA sequence (Blastnetwork). To recognize the  $\text{GABA}_{\text{B}}$  receptor, sense and anti-sense probes specific for the  $\text{GABA}_{\text{B}1\text{a}}$  receptor ( $5' > \text{TTC ACC TGG TCG CGA GTC AAG CCA CGG TAC CTG ATG CCA C} < 3'$ ) and a pan probe recognizing both  $\text{GABA}_{\text{B}1\text{a}}$  and  $\text{GABA}_{\text{B}1\text{b}}$  receptor ( $5' > \text{GCC CTC CAC CGC CTC GGT CAT TTC TTC CAC TGT ACA ATT G} < 3'$ ) were used following a protocol described previously in some detail (Wüllner et al., 1997). Probes were 3' end labeled with  $^{35}\text{S}$  (Hartmann Analytic, Braunschweig, Germany) using a terminal transferase kit (NEN 100 kit, Dupont-NEN). Frozen sections were dried at room

temperature, postfixed in 4% paraformaldehyde, pre-washed in phosphate buffer and alcohols and dried at room temperature. Sections were overnight hybridised at  $40^{\circ}\text{C}$  with  $10000\ \text{dpm}/\mu\text{l}$  probe. Following hybridisation the slides were washed in standard saline-sodium citrate (SSC) and ethanol, airdried then exposed to  $\beta$ -max film (Amersham) for 18 days and developed in D19 developer (Kodak). Films were analysed using the MCID M4 image processing system (Imaging Research, St. Catherine's, Ontario). The optical density of four sections per animal per region were averaged for each probe.

### 2.6. Statistical analysis

Rotational behaviour (sum of rotations in 60 min) was statistically evaluated using a one way ANOVA followed by a Tukey test. The results of the *in situ* hybridisation were evaluated using a two-tailed, paired Student's *t*-test.

## 3. Results

### 3.1. Rotational behaviour

Microinjection of 2-hydroxysaclofen into the left VM of 6-OHDA lesioned rats immediately induced rotational behaviour (Fig. 1). The effect was dose-dependent and statistically significant levels were obtained with 100 nmol 2-hydroxysaclofen. Microinjections into the nuclei surrounding the VM, the zona incerta or ventrolateral thalamic nucleus (VL), did not result in

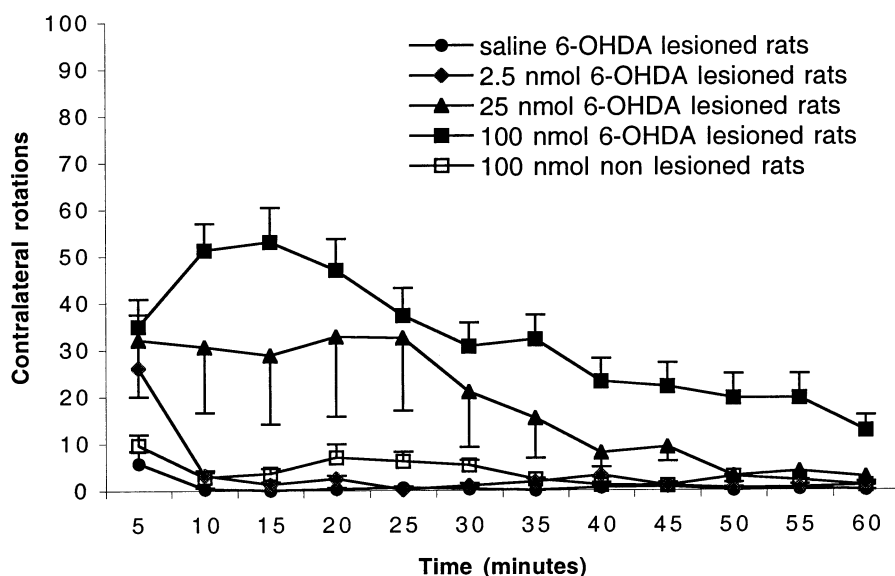


Fig. 1. The effects of microinjection of 2-hydroxysaclofen into the left ventromedial thalamus of 6-hydroxydopamine lesioned rats (2.5, 25 and 100 nmol) and intact rats (100 nmol) on rotational behaviour. Data are presented as means, error bars indicate standard error of the mean ( $n = 5-7$ ). For the purpose of representation ipsilateral rotations are not shown. Ipsilateral rotations did not differ from saline injections.

Table 1  
The effect of microinjection of GABA<sub>B</sub> receptor antagonists into the (ipsilateral) VM of unilateral 6-OHDA lesioned rats on rotational behaviour<sup>a</sup>

Compound	Dosis ( <i>n</i> )	Rotations ipsilateral (SEM)	Rotations contralateral (SEM)
Saline	(7)	9.75 (7.44)	7.00 (2.97)
2-hydroxysaclofen	0.025 nmol (5)	4.92 (2.200)	23.00 (5.23)
	0.25 nmol (4)	3.20 (1.60)	21.25 (6.68)
	2.5 nmol (5)	2.00 (2.35)	34.00 (10.08)
	25 nmol (5)	2.80 (1.34)	177.60 (92.53)
	100 nmol (7)	5.57 (2.43)	254.29 (72.49) <sup>b</sup>
SCH 50911	0.025 nmol (3)	1.95 (1.10)	3.90 (2.30)
	25 nmol (5)	4.50 (2.60)	3.20 (1.80)
	100 nmol (3)	3.00 (2.00)	2.00 (1.30)
	1 μmol (2) <sup>c</sup>	1.50 (1.41)	2.50 (2.30)
	5 μmol (2) <sup>c</sup>	2.50 (2.12)	2.00 (1.30)
CGP 56433	0.025 nmol (4)	1.5 (1.29)	3.25 (1.71)
	2.5 nmol (3)	4.00 (2.65)	4.33 (2.10)
	12.5 nmol (2) <sup>c</sup>	3.50 (2.00)	3.00 (2.90)
CGP 55854	0.25 nmol (5)	3.40 (1.90)	4.50 (2.10)
	2.5 nmol (4)	2.75 (1.55)	3.00 (2.90)

<sup>a</sup> Data represent total number of rotations in 60 min.

<sup>b</sup>  $P < 0.05$  versus saline treated rats.

<sup>c</sup> Convulsant dosis.

contralateral rotations (data not shown). To test whether the effect of 2-hydroxysaclofen was specific for rats with nigrostriatal lesions we injected 2-hydroxysaclofen (100 nmol) into the VM of rats without a nigrostriatal lesion which resulted in a significantly lower rotational response compared to injection of 100 nmol 2-hydroxysaclofen in 6-OHDA lesioned rats ( $P < 0.05$ ). Microinjection of SCH 50911 (0.025, 25, 100 nmol), CGP 55845 (0.25 and 25 nmol) and CGP 56433 (0.025 and 2.5 nmol) into the VM had no effect on rotational behaviour or body posture in nigrostriatal lesioned rats (Table 1). At higher doses, SCH 50911 (100 and 5 μmol) and CGP 56433 (12.5 nmol) induced convulsions without affecting rotational behaviour or body posture. At these doses SCH 50911 and CGP 56433 also produced convulsions when injected into the VL and the VM of rats without nigrostriatal lesions. Since higher doses of SCH 50911 and CGP 56433 were proconvulsant we decided not to test higher doses of CGP 55845.

### 3.2. GABA<sub>B</sub> receptor mRNA expression

GABA<sub>B</sub> receptor mRNA expression was found throughout the thalamus and basal ganglia. In the thalamus strong signals for the GABA<sub>B</sub> pan probe were found in the VM, VL, laterodorsal thalamic nucleus (LD), medial geniculate nucleus (MGN), lateral geniculate nucleus (LGN) and lateral habenula (LHB) (Fig. 2). The distribution and signal for the GABA<sub>B</sub>1a probe was similar to that of the GABA<sub>B</sub> pan probe with exception of the medial geniculate nucleus (MGN), where a weaker signal was detected for the GABA<sub>B</sub>1a probe (Fig. 2B, D). In unilateral nigrostriatal lesioned

rats the signal for both the GABA<sub>B</sub>1a and GABA<sub>B</sub> pan probe was reduced in the left, lesioned substantia nigra compacta (Fig. 2C,D). In the VM, VL, SNr, GP and caudate putamen (CP) of nigrostriatal lesioned rats, no differences in optical density were found between the side ipsilateral to the lesion and the side contralateral to the lesion (Fig. 3). No labeling was observed in sections processed with sense RNA probes.

## 4. Discussion

This study was performed to test the hypothesis that the abnormalities of basal ganglia output that lead to parkinsonism are mediated by increased GABA<sub>B</sub> inhibition in the motor thalamus. This hypothesis predicts that blockade of GABA<sub>B</sub> receptors by local microinjection of specific GABA<sub>B</sub> antagonists into the motor thalamus, the VM in rats, will have an antiparkinsonian action. In addition, one would expect that excessive inhibition mediated by GABA<sub>B</sub> receptors in the VM would result in an altered receptor expression within this nucleus. In contrast to the predictions of this hypothesis, blockade of GABA<sub>B</sub> receptors in the VM of nigrostriatal lesioned rats with the novel high affinity GABA<sub>B</sub> antagonists SCH 50911, CGP 56433 and CGP 55845 did not affect rotational behaviour. In contrast, 2-hydroxysaclofen induced contralateral rotations. The fact that antagonists with a high affinity for the GABA<sub>B</sub> receptor failed to induce rotational behaviour raises the question whether the effects of 2-hydroxysaclofen are mediated at GABA<sub>B</sub> receptors on thalamocortical neurons. In vitro studies have demonstrated presynaptic actions of 2-hydroxysaclofen (Emri

et al., 1996), although in another study no clear-cut evidence was found for the existence of presynaptic GABA<sub>B</sub> receptors in the rat VM (Timmerman and Westerink, 1997). Alternatively, 2-hydroxysaclofen can act as a partial agonist and influence GABA<sub>A</sub> receptor mediated transmission or GABA release (Caddick et al., 1995; Churchill et al., 1996). Such action of 2-hydroxysaclofen could result in a reduction of GABA release in the synaptic cleft, relieve thalamocortical neurons in the VM from their tonic GABAergic inhibition and thus result in contralateral rotations. The fact that injection of 2-hydroxysaclofen into the VM of rats with an intact nigrostriatal pathway also induced contralateral rotations, though significantly lower as in nigrostriatal lesioned rats, could indicate that GABA release is indeed altered in unilateral nigrostriatal lesioned rats. We observed convulsions after application of high affinity GABA<sub>B</sub> receptor antagonists into different thalamic nuclei. Although delayed convulsions were observed in some animals, these did not seem to be the result of leakage from the microinfusion solution into the cortex since in most animals convulsions started directly after application of GABA<sub>B</sub> antagonists. Recent studies reported similar findings after systemic and local application of high affinity GABA<sub>B</sub> receptor antagonists in cortex and limbic structures of intact mice and rats, yet the mechanism responsible for GABA<sub>B</sub> antagonist induced convulsions remains unclear (Badran et al., 1997; Vergnes et al., 1997).

In addition to our behavioural studies we investigated the expression of GABA<sub>B</sub> receptor mRNA in nigrostriatal lesioned rats. GABA<sub>B</sub> receptor mRNA

expression was found throughout the basal ganglia and thalamus and the distribution of the splice variants is in accordance with recent reports (Bisschof et al., 1997; Kaupmann et al., 1997). Within the basal ganglia, a reduction of GABA<sub>B</sub> receptor expression in the SNc on the lesioned (left) side was found, confirming that GABA<sub>B</sub> receptors are present on SNc neurones where they may play a role in providing feedback of striatal dopamine release (Engber and Nissbrandt, 1993). A unilateral nigrostriatal lesion of the SNc did not alter GABA<sub>B</sub> receptor expression at the postsynaptic transcriptional level in the VM. A recent study demonstrated that thalamic GABA<sub>B</sub> receptor expression can be activity dependent, since monocular deprivation reduced the level of GABA<sub>B</sub> receptor expression in the monkey lateral geniculate nucleus (Munoz et al., 1998). The rat practically lacks GABAergic interneurons in the VM which means that the signal for GABA<sub>B</sub> receptor mRNA is localized in projection neurones (Saywer et al., 1994). It must be noted that the VM not only sends efferents to the cortex but also to the striatum and reticular thalamic nucleus (Herkenham, 1979; MacLeod et al., 1980).

The current concept of basal ganglia function implies that GABAergic overinhibition of thalamocortical neurones results in akinesia, the cardinal feature of Parkinson's disease. However, lesions of motor parts of the thalamus do not result in akinesia but in the contrary improve the symptoms of Parkinson's disease (Marsden and Obeso, 1994; Lang and Lozano, 1998). Similarly, studies in the rat showed that unilateral or bilateral lesions of the VM in rats do not result in hypomotility

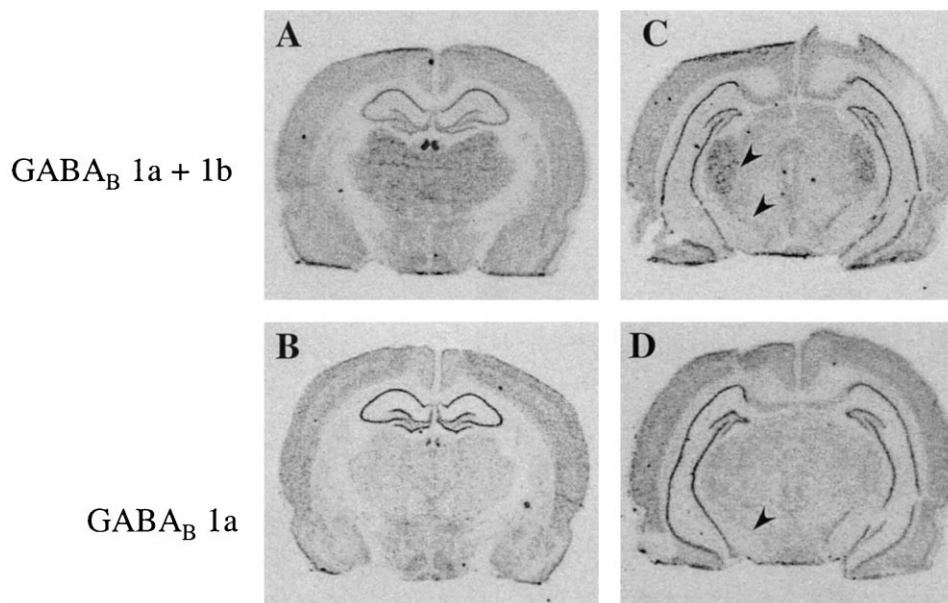


Fig. 2. Autoradiograms of sections from 6-OHDA lesioned rats hybridised with GABA<sub>B</sub> pan probe (A, C) and GABA<sub>B</sub>1a probe (B, D) at the level of the ventromedial thalamus (A, B) and substantia nigra (C, D). Note the absence of signal in the left, lesioned substantia nigra compacta (C, D) and higher expression of the pan probe in the medial geniculate nucleus (arrowheads).

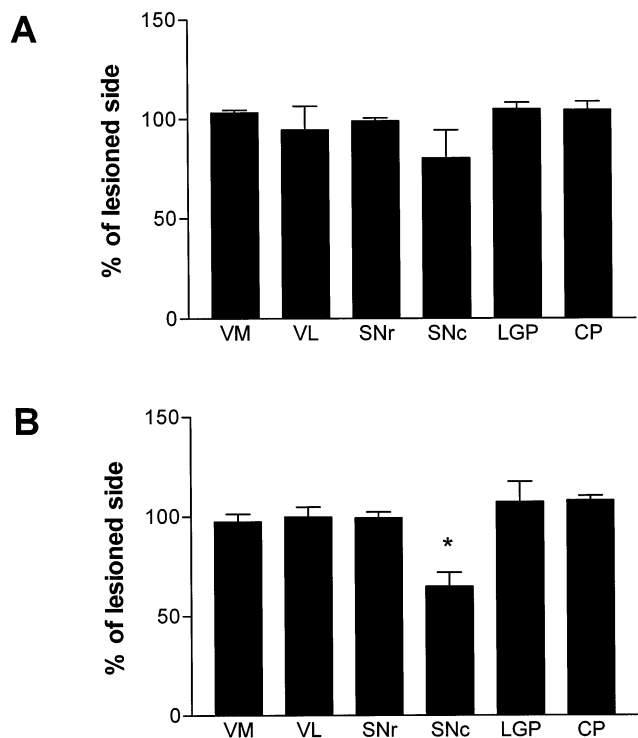


Fig. 3. Quantitative optical density measurements of the hybridization signals for the GABA<sub>B</sub> 1a probe (A) and GABA<sub>B</sub> pan probe (B) in the basal ganglia and thalamus of 6-OHDA lesioned rats. For the means of representation data are shown as percentage of the optical density on the left side compared to that on the right side. In control animals there were no side to side differences. Data represent mean optical density and error bars indicate standard deviation ( $n = 5-7$ ). Statistical analysis was performed on raw data, not on percentages. \*  $P < 0.05$ . VM, ventromedial thalamic nucleus; VL, ventrolateral thalamic nucleus; SNr, substantia nigra pars reticulata; SNc, substantia nigra pars compacta; LGP, lateral globus pallidus; STN, subthalamic nucleus; CP, caudate putamen.

or catalepsy (Starr and Summerhayes, 1983b). It has been suggested that the electrophysiological basis that underlies this phenomenon could be an altered, oscillatory firing pattern of thalamic neurones in response to increased GABAergic inhibition (Vitek et al., 1994; Wichman and DeLong, 1996). Such oscillatory activity of thalamocortical neurones has originally been postulated as the underlying mechanism of tremor but has been less associated with akinesia (Buszáki et al., 1990; Paré et al., 1990). Periodic or tonic GABAergic stimulation of thalamocortical cells *in vitro* activates GABA<sub>B</sub> receptors which results in hyperpolarisation of the cell membrane and oscillatory activity associated with low threshold calcium spikes (Buszáki, 1991; Crunelli and Leresche, 1991; Williams et al., 1995; Destexhe, 1998). Increased basal extracellular GABA levels have indeed been measured in the ventrolateral thalamus of rats with spontaneous absence seizures suggesting that *in vivo* spike-and-wave discharges could also be the consequence of GABAergic overinhibition of thalamic neurones (Richards et al., 1995). GABAergic transmission

plays an important role in motor behaviour since bilateral application of GABA<sub>A</sub> and GABA<sub>B</sub> agonists into the VM induce catalepsy (DiChiara et al., 1979; Klockgether et al., 1985, 1986; Wüllner et al., 1987). Bilateral application of the GABA<sub>A</sub> antagonist bicuculline into the VM of rats produces pronounced hyperactivity whereas rotational behaviour can be induced following unilateral application of bicuculline into the VM of intact animals (Starr and Summerhayes, 1983a). A unilateral nigrostriatal lesion in rats should result in increased GABAergic output of the basal ganglia to the VM on the side ipsilateral to the lesion and blockade of GABAergic transmission should result in stronger rotational behaviour, which we have indeed observed with 2-hydroxysaclofen. Evidence for an increased basal ganglia output includes increased cytochrome oxidase, a marker for metabolic activity, in the SNr and MGP and increased burstfiring in the SNr (MacLeod et al., 1990; Porter et al., 1994). Increased GABAergic neurotransmission could lead to a down regulation of (postsynaptic) GABA<sub>B</sub> receptors, however we did not find changes in GABA<sub>B</sub> receptor expression in the ventromedial thalamus of rats with unilateral nigrostriatal lesions, indicating that no changes of GABA<sub>B</sub> receptors occur at the transcriptional level of thalamic projection neurones.

In contrast to the predictions of our hypothesis, specific, high-affinity GABA<sub>B</sub> receptor antagonists did not induce contralateral rotations in 6-OHDA-lesioned rats after microinjection into the VM. However, the partial GABA<sub>B</sub> antagonist 2-hydroxysaclofen induced rotational behaviour, i.e. alleviated parkinsonian symptoms, suggesting that the effects of 2-hydroxysaclofen are either mediated at presynaptic GABA<sub>B</sub> receptors or through reduced GABA release. In addition, a 6-OHDA lesion of the nigrostriatal dopaminergic system did not affect GABA<sub>B</sub> receptor expression in the VM. These results make it improbable that thalamocortical GABA<sub>B</sub> receptors play an important role in the pathophysiology of parkinsonism. Therefore, GABA<sub>B</sub> receptors do not appear to be a promising target for novel antiparkinsonian drugs.

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