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N-Methyl-D-Aspartate Receptor Activation Interacts with Electrical High Frequency Stimulation in the Rat Caudate Nucleus in vitro and in vivo

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ABSTRACT

Electrical high frequency stimulation is used as treatment for motoric- as well as psychomotoric disorders in various regions of the brain. The biochemical mechanisms of the action of high frequency stimulation underlying the effective results are unclear. Previous studies have demonstrated that electrical 130 Hz stimulation was capable of increasing local γ-aminobutyric acid outflow in the rat caudate nucleus when neuronal pre-activation was present. In the present study, the local effect of electrical high frequency stimulation on γ-aminobutyric was reviewed under conditions of N-methyl-D-aspartate receptor activation. Using in vitro superfusion experiments with tissue slices from the rat caudate nucleus, and co-localized in vivo microdialysis and electrical 130 Hz stimulation of the caudate nucleus in awake and freely moving rats, we can conclude that N-methyl-D-aspartate receptor activation was able to modulate γ-aminobutyric acid outflow induced by electrical high frequency stimulation.

INTRODUCTION

Deep brain stimulation (DBS) is nowadays a widely applied therapy for intractable neurological disorders, several movement disorders and in particular for advanced Parkinson's disease (PD) [1-4]. Electrical high frequency stimulation with 130 Hz in the deep brain region of the subthalamic nucleus has been used to treat parkinsonian motor symptoms with remarkable success [5]. In the subgenual cingulate cortex, the anterior internal capsule and the thalamic centre

median/parafascicular nucleus, electrical high frequency stimulation (HFS) is used to treat depression, obsessive-compulsive disorder and Tourette's syndrome [6-8].

There is evidence that γ-aminobutyric acid (GABA) neurons play an essential role for the efficacy of electrical high frequency stimulation [9-12]. In previous studies, electrical 130 Hz stimulation of vital slices of the rat caudate nucleus in superfusion chambers, led to increased extracellular GABA outflow from GABAergic medium spiny neurons, depending on a GABA transporter mechanism after neuronal pre-activation by the voltage-gated sodium channel opener veratridine [13, 14]. Increased GABA outflow, due to electrical 130 Hz stimulation, was also observed in the caudate nucleus of awake and freely moving rats. However, this was not found in anaesthetized animals [15].

Based on this background, the aim of the current study was to investigate N-methyl-D-aspartate (NMDA) receptor activation as intrinsic neuronal excitation, related to the effect of electrical high frequency stimulation.

MATERIALS AND METHODOLOGY

All procedures with animals were reviewed and approved by the University of Luebeck and the Ministry for Agriculture, the Environment and Rural Areas, Schleswig-Holstein, Germany, and were conducted in accordance with the NIH guide for the Care and Use of laboratory animals.

Male Wistar rats were used and housed separately under standard lightning conditions (12 h light-dark cycle, lights on 06:00 am), 22°C and 40% humidity with free access to food and water.

In vitro experiments:

For *in vitro* slice experiments, rats (250-300 g) were decapitated after deep carbon dioxide anesthesia. Brains were removed and immersed in ice-cold artificial cerebrospinal fluid (aCSF). The aCSF was composed of 125 mM NaCl, 25 mM $NaHCO_3$, 2 mM $CaCl_2$ x $2H_2O$, 5 mM KCl, 1.29 mM KH_2PO_4 (Merck Chemicals), 10 mM glucose (Fluka), 0.1 mM ascorbic acid (Sigma-Aldrich), dissolved in HPLC grade H_2O (J.T. Baker), and adjusted to pH 7.4 (Butcher et al., 1988; Thümen et al., 2002). When indicated, 1.14 mM $MgSO_4$ x $7H_2O$ (Merck Chemicals) was added.

The caudate nucleus was dissected, and 300 µm tissue slices were obtained using a tissue sectioner (Tissue Chopper, McIlwain, CA). The slices were transferred into superfusion chambers, equipped with platinum electrode wires on both sides. Artificial CSF, purged with 95% oxygen and 5% carbon dioxide was pumped through the chamber at 35 °C and at a flow rate of 250 µl/min.

Basal GABA and glutamate outflow was established after a 40 min equilibration period. Superfusion samples were initially collected every 10 min. The first four 10 min fractions (10 – 40 minutes of superfusion time) were taken as basal outflow. From 40 minutes on, samples were collected every 5 minutes. Electrical high frequency stimulation was performed, by applying monophasic, rectangular high-frequency pulses (hf-pulses) with 130 Hz, 0.6 mA constant current and 60 µs duration, for 10 minutes (40 – 50 minutes of superfusion time). Pulses were applied via platinum electrodes in the superfusion chamber. During electrical stimulation, the NMDA receptor agonist (RS)-(tetrazol-5-yl) glycine (RS-TG) was added to the aCSF when indicated. After 80 minutes of superfusion, the experiment was stopped, brain slices were removed from chambers and tissue wet weight was obtained. In the present study, the wet weight of slices in each of the chambers showed no significant differences. The mean wet weight was 2.8 ± 0.2 mg.

In vivo experiments:

For *in vivo* microdialysis experiments with co-localized and simultaneous high frequency stimulation, a double tube guiding cannula was prepared to hold the probe tips in place at 0.5 mm distance and 20° angle [15]. Using a stereotaxic frame for small animals (Stoelting Co., US) and standard stereotaxic method, the double tube guide cannula was implanted into the ventromedial part of the rat caudate nucleus [16]. Prior to the surgery, rats were pre-anesthetized with isoflurane and then injected with 80 mg/kg ketamine (Ketavet®, Pfizer) and 1 mg/kg xylazine (Rompune®, Bayer) *i.p.* The double guide cannula was placed -0.26 mm *anterior-posterior* and -3.0 mm *medial-lateral* relative to bregma and - 3.0 mm *dorsal-ventral* from the dural surface, and fixed to the skull with dental resin.

After a three-day recovery period,

microdialysis experiments were performed. A microdialysis probe with a 4 mm membrane (CMA/11 4 mm, CMA, Sweden) was inserted into the medial guide, and, a modified bipolar concentric stimulation electrode, with a 45° beveled tip (CBCBG30, FHC, US) was inserted into the lateral guide of the double tube guide cannula. For all experiments, the microdialysis probe was continuously perfused with aCSF resembling the composition used in the *in vitro* experiments. A flow rate of 1.2 µl/min was used. The NMDA receptor agonist RS-TG was added to the aCSF in different concentrations as indicated. Basal GABA and glutamate outflow was established after a 120 min equilibration period. Samples were collected every 20 minutes in 15 consecutive fractions. For each fraction, 24 µl of dialysate was collected into 5 µl 3.3 % perchloric acid (Merck Chemicals). After equilibration, the first three fractions (20 – 60 min perfusion time) were taken as basal outflow. Electrical high frequency stimulation was performed twice, for 30 minutes each, by applying monophasic, rectangular pulses with 124 Hz, 0.5 mA constant current and 60 µs duration from 60 – 90 minutes and 150 – 180 minutes of perfusion time respectively. Using a stimulus isolator (Isostim A320, WPI, US), HF-pulses were applied via the bipolar concentric platinum/iridium electrode with the electric field pointing to the microdialysis membrane [17]. The experiment was stopped after 240 minutes of sampling. The probes were removed and the animals were decapitated under deep carbon dioxide anesthesia.

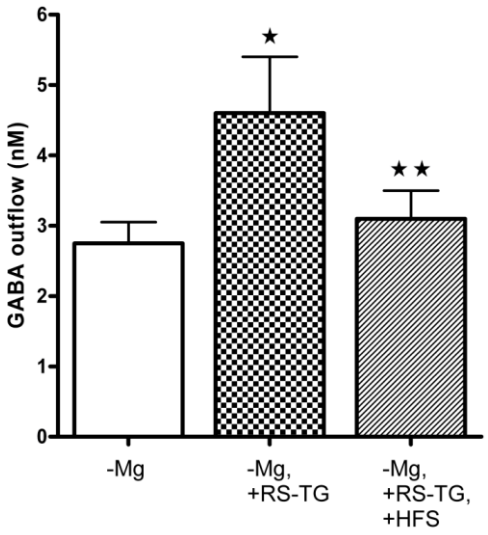
All collected samples were stored at -30 °C, and subsequently measured by means of high performance liquid chromatography (HPLC), with electrochemical detection, after pre-column derivatisation with o-phthaldialdehyde sulphite [15]. The HPLC system consisted of a C18 column (Eurospher RP 18.5 µm, column size 250 x 4 mm with a pre-column 35 x 4 mm). The mobile phase (0.1 M sodium phosphate buffer, pH 4.5, containing 0.5 mM EDTA and 25% methanol), previously degassed by helium, was pumped at a flow rate of 1.0 ml/min. GABA and glutamate were detected electrochemically using a glassy carbon electrode set at a potential of 900 mV relative to an Ag/AgCl reference electrode. GABA and glutamate outflow were expressed in nM or as % of control value ± standard error of the mean (SEM). Differences between the measured treatment samples and their corresponding controls were tested with a one-way analysis of variance and follow up t-tests for pairwise comparisons as indicated.

RESULTS AND OBSERVATIONS

In vitro experiments:

In the superfusion experiments, basal neurotransmitter outflow from striatal slices was 2.73 ± 1.08 nM GABA and 34.4 ± 9.8 nM glutamate in the superfusate. Addition of the NMDA agonist RS-tetrazol-5yl-glycine (RS-TG, 10 µM) had no significant effect on basal GABA outflow in presence of 1.14 mM magnesium ions in the aCSF. In contrast, RS-TG (10 µM) was able to significantly induce an increase of extracellular GABA levels to 4.58 ± 0.96 nM when magnesium ions were omitted from the incubation medium (Figure 1). In the presence of magnesium ions, application of HF-pulses did not modify GABA outflow in the absence or presence of RS-TG. However, in magnesium-free aCSF, HF-pulses significantly diminished the RS-TG-induced GABA outflow from 4.58 ± 0.96 nM to 3.06 ± 0.49 nM (Figure 1). Glutamate outflow remained unchanged in the presence of RS-TG with or without HF-pulses.

Figure 1: The effect of the NMDA receptor agonist RS-tetrazol-5yl-glycine (RS-TG, 10 µM) on GABA outflow from slices of the rat caudate nucleus (nM ± SD), in the absence and presence of electrical high frequency stimulation. Magnesium ions were omitted from the incubation medium.



★significant when compared to –RS-TG ($p < 0.01$).
★★significant when compared to –HFS ($p < 0.01$).

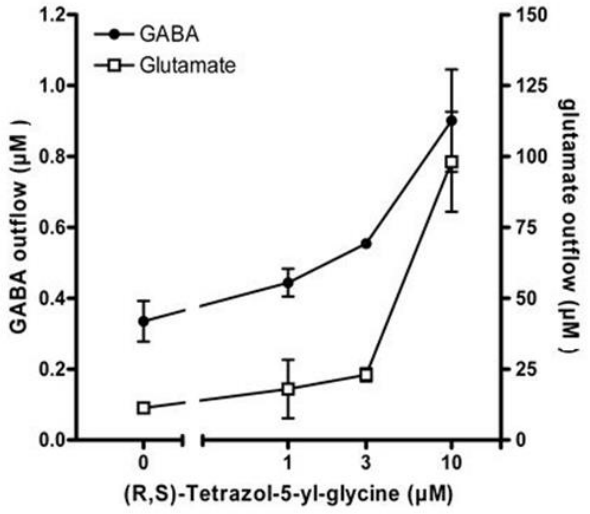
The following experiments with the NMDA receptor coactivator glycine were performed in presence of the glycine receptor blocker strychnine (10 µM). Glycine (10 µM) did not significantly modulate the RS-TG-evoked increase of GABA outflow in absence of magnesium ions in the aCSF (Table 1). Additionally, the inhibitory effect of HF-pulses on RS-TG evoked GABA outflow remained unchanged in presence of glycine (Table 1). In all these experiments, no significant modifications of glutamate outflow were observed.

Table 1: GABA outflow in the presence of RS-TG (10 µM) or RS-TG + glycine (10 µM) in the absence and presence of hf-pulses.

	Basal	RS-TG	RS-TG + glycine
- hf-pulses	100 % ± 12.4	168 % ± 20.9	145 % ± 6.9
+ hf-pulses	100 % ± 19.1	121 % ± 16.1 *	117 % ± 13.2 *

* p<0.05 when compared to absence of hf-pulses. In aCSF, magnesium ions were omitted. Glycine experiments were performed in the presence of the glycine receptor blocker strychnine (10 µM). Values are expressed as in % of basal values (= 100 %) ± SEM.

Figure 2: The effect of RS-tetrazol-5yl-glycine (RS-TG) on GABA (left axis) and glutamate (right axis) outflow in awake and freely moving rats (µM ± SD). Basal outflow was calculated from samples at 20, 40, 60 min sampling time.

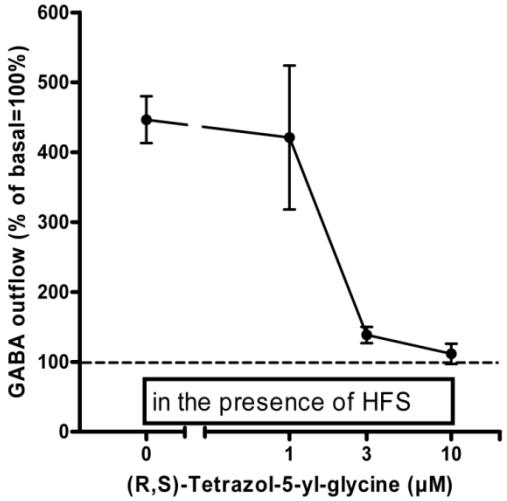


In vivo experiments:

In the microdialysis experiments, neurotransmitter concentrations were 0.34 ± 0.06 µM GABA and 11.3 ± 1.9 µM glutamate in the dialysate under basal conditions (Figure 2). GABA as well as glutamate outflow markedly and dose-dependently increased when the NMDA receptor agonist RS-TG was added to the perfusion fluid. At a maximum dose of 10 µM RS-TG, GABA outflow increased up to 270 ± 17 % compared to basal values, and glutamate levels increased to approximately 870 ± 155 % (Figure 2).

Under basal conditions, electrical high frequency stimulation with 124 Hz enhanced extracellular GABA levels to nearly 450 ± 33 % as described earlier [15]. When RS-TG was co-administered with electrical high frequency stimulation, GABA levels dose-dependently decreased. In the case of 10 µM RS-TG in the perfusion medium, high frequency stimulation did not further enhance GABA outflow (Figure 3). In all experiments, electrical high frequency stimulation did not modify glutamate outflow.

Figure 3: Co-administration of RS-tetrazol-5yl-glycine (RS-TG) and electrical 124 Hz stimulation with 0.5 mA (HFS). Normalized mean values and standard errors are displayed as percentage of basal GABA outflow (=100%).



DISCUSSION

Despite various discussions on the mechanisms of electrical high frequency stimulation (HFS), there is evidence that GABAergic neurons play an essential role in its efficacy [12, 14, 15, 18]. Against the background of controversial effects on neuronal activity, different co-factors have to be considered. Li et al. (2004, 2006) demonstrated that HFS increased GABA outflow from slices of the rat caudate nucleus only in presence of the voltage-gated sodium channel opener veratridine, mimicking depolarisation [13, 14]. Besides in this *in vitro* model of pre-activation, it has been demonstrated that an endogenously activated neuronal state is

also required for significant HFS effects *in vivo*. In microdialysis experiments, Hiller et al. (2007) showed that HFS evoked GABA outflow in awake and active rats, but not during anaesthesia with sodium pentobarbital [15]. These observations imply that HFS is not effective when GABAergic neurons are resting. Thus, the aim of this study was to examine if NMDA receptor stimulation activates GABA neurons similar to veratridine, facilitating the effect of HFS on GABA outflow in the rat caudate nucleus. The NMDA subtype of ionotropic glutamate receptors plays a key role in numerous physiological and pathological processes of the brain. Strong evidence for presynaptic NMDA receptors on GABAergic neurons of the rat caudate nucleus has been provided by previous studies of Schoffelmeer et al. (2000) and Hanania and Johnson (1999) [19, 20]. These studies demonstrated that NMDA receptor-mediated GABA outflow and proposed a reversal of the GABA transporter due to intracellular Na^+ increase after NMDA receptor induced cat-ion channel opening.

In our slice superfusion model, the NMDA receptor agonist RS-tetrazol-5yl-glycine (RS-TG) led to an increase of GABA ouflow, only in magnesium-free superfusion buffer. Independent of the presence of magnesium, glutamate outflow was unaffected by RS-TG. The important biophysical properties of the NMDA receptor channel that determine its specific involvement in synaptic processes are a high permeability for Ca^{2+} ions and a voltage-dependent block by magnesium ions [21]. As a result of a combination of these features, activation of the NMDA receptor at voltages near rest does not lead to Na^+ and Ca^{2+} influx, as the channels are blocked by endogenous magnesium ions. Only significant depolarization leads to the reverse of magnesium block and allows modulation of incoming inputs [22-24]. Taken together, our *in vitro* findings on magnesium support NMDA receptor related effects of RS-TG on GABAergic striatal neurons as reported by Krebs et al. 1994 [25].

Under *in vivo* conditions, RS-TG added in the perfusion medium led to a marked increase in GABA as well as glutamate levels. In microdialysis experiments in freely moving rats, the caudate nucleus was embedded in a complete and functional neuronal network. It received various excitatory afferents, mainly from the cortex. Therefore, experimental magnesium depletion might not be necessary to remove the magnesium block from the NMDA receptor's channel pore. The intrinsic activity of the network might be sufficient to depolarize the postsynaptic membrane and facilitate NMDA receptor mediated effects. Increased GABA outflow due to NMDA receptor activation could be observed *in vivo* when afferent projection neurons were intact, and *in vitro*, when projections were cut for a clean slice preparation, and might therefore be related to NMDA receptors located on striatal GABAergic medium spiny neurons [26, 27].

Even when the superfusion liquid was free of magnesium, RS-TG did not modify extracellular glutamate levels *in vitro*. In the *in vivo* microdialysis model, glutamate outflow increased dose-dependently when RS-TG was administered. The nature of intrastriatal glutamate release evoked by NMDA receptor activation is controversially discussed, especially since glutamate autofacilitation might induce excitotoxic effects. However, NMDA receptor activation that may mediate striatal glutamate outflow *in vivo* has been reported for other NMDA receptor agonists [28, 29]. Beside autofacilitation through presynaptic NMDA receptors located on striatal glutamatergic synapses, NMDA receptor dependent glutamate outflow might also be due to indirect activation of striatal NK1 and DA receptors or extrastriatal loops [30]. Our study supports the suggestion of an extrastriatal mechanism since in slices with missing excitability of extrastriatal loops, NMDA receptor mediated glutamate release could not be observed. NMDA receptor mediated glutamate outflow could only be found *in vivo* but was not modified by electrical high frequency stimulation. In contrast to glutamate, NMDA receptor stimulation by the agonist RS-TG markedly blocked GABAergic activation induced by HFS *in vivo* [15]. Since HFS focussed on striatal GABAergic medium spiny neurons [11, 12, 14], interaction between the NMDA receptor agonist RS-TG and HFS may also be a local intrastriatal phenomenon. Taken together, we would propose that HFS effects can be modulated by NMDA receptor activation in the caudate nucleus.

Our findings confirm the conclusion drawn by Li et al. 2006, Mantovani et al. 2009, and Feuerstein et al. 2011, that GABA outflow induced

by HFS acts on GABAergic neurons [11, 12, 14]. In the rat caudate nucleus this effect can be modulated by NMDA receptor activation probably located on medium spiny neurons.

CONCLUSION

Electrical high frequency stimulation is used to treat several movement disorders and other treatment resistant neurological disorders. The mechanisms underlying the effects of high frequency stimulation are still unclear. The effective role of GABAergic neurons in the effects of high frequency stimulation has been discussed in previous research works. We discussed the modulation of the HFS effect by NMDA receptor activation located in the rat caudate nucleus. In summary, the results from this study indicated that the HFS effect can be modulated by the NMDA receptor activation, which is probably located on the medium spiny neurons of the rat caudate nucleus. These results are important to have a better understanding of the biochemical mechanism underlying the HFS effect.

LIST OF ABBREVIATIONS

GABA – γ-aminobutyric acid

NMDA – N-methyl-D-aspartate

aCSF – artificial cerebrospinal fluid

hf-pulses – high-frequency pulses

RS-TG – (RS)-(tetrazol-5-yl)glycine

HPLC – high performance liquid chromatography

HFS – high frequency stimulation

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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