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Electrical high frequency stimulation of the caudate nucleus induces local GABA outflow in freely moving rats

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Abstract

Electrical high frequency stimulation of the globus pallidus internus or the subthalamic nucleus has beneficial motor effects in advanced Parkinson's disease. The mechanisms underlying these clinical results remain, however, unclear. From previous in vitro studies it is proposed that the γ-aminobutyric acid (GABA) system is involved in the effectiveness of electrical high frequency stimulation (HFS). In these experiments, we developed an in vivo model that allows for simultaneous and collocated microdialysis and HFS by electrical pulses of 124 Hz in the caudate nucleus of freely moving rats. GABA and glutamate outflow were sampled by microdialysis technique and quantified after pre-column *o*-phthaldialdehyde sulphite derivatization using HPLC with electrochemical detection. As the most outstanding result, we could demonstrate that high frequency stimulation significantly increased basal GABA outflow without affecting glutamate levels in freely moving rats.

Keywords: Electrical high frequency stimulation; Deep brain stimulation; γ-Aminobutyric acid; Microdialysis; Freely moving rats

1. Introduction

Deep brain stimulation (DBS) by means of implanted electrodes has become a well accepted technique for treatment of several movement disorders (Gross and Lozano, 2000; Tronnier et al., 2002). In particular for an advanced state of Parkinson's disease electrical high frequency stimulation (HFS) with 130 Hz of the globus pallidus internus (GPi) or the subthalamic nucleus (STN) has proved to alleviate resting tremor and rigidity, and very effectively reduce L-Dopa induced dyskinesia (Benabid et al., 2001; Vingerhoets et al., 2002). The mechanisms of the molecular and pharmacological actions of HFS are, however, still unknown.

The effectiveness of HFS has been described by various mechanisms, ranging from blockage of depolarisation to stimulation-evoked release of γ-aminobutyric acid (GABA) or stimulation of GABA receptors (Benazzouz and Hallett, 2000; Dostrovsky and Lozano, 2002; Vitek, 2002).

Data of in vitro studies provide initial evidence that HFS not only requires $GABA_A$-receptors but also intact GABAergic nerve terminals coupled to $GABA_A$-receptors to achieve an inhibitory effect (Li et al., 2004). These results also indicate that HFS has a specific effect on GABAergic neuronal terminals resulting in an enhancement of extracellular GABA. In vitro, this effect is most likely due to an inhibitory effect of HFS on the GABA uptake system, rather than to the stimulation of a vesicular GABA release from GABAergic neurons, which are both associated with the presynaptic GABAergic physiology (Li et al., 2004, 2006). Therefore, we developed an in vivo model that allows for simultaneous and collocated microdialysis and HFS in the caudate nucleus of alert and active rats, which are stimulated by electrical pulses of 124 Hz, to analyse neurotransmitters under HFS. Although for DBS the GPi and STN are the most important structures (Samuel et al., 1988), these two regions are very small in rats and therefore not suitable for our in vivo experiments. The large-sized caudate nucleus was employed due to the high density of striatal GABAergic neurons, but not because its linkage to Parkinson's disease.

2. Material and methods

All procedures with animals were reviewed and approved by the University of Lübeck and the "Ministerium für Umwelt,

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Natur und Forsten des Landes Schleswig-Holstein, Germany", and were conducted in accordance with the NIH guide for the Care and Use of laboratory animals.

Male Wistar rats (400 g) were used in this study. Rats were housed singly under standard lightning (12 h light–dark cycle, lights on 06:00 a.m.), temperature (22 °C), and humidity (40%) conditions, and were allowed free access to food and water.

For guide cannula implantation, rats were pre-anesthetized with CO_2 followed by full anaesthesia with sodium pentobarbital (53 mg/kg i.p.). Using standard stereotaxic techniques, an intracerebral double guide cannula was placed just above the right caput nuclei caudati and fixed to the skull with dental cement. The double guide cannula consisted of a commercial microdialysis cannula (CMA/11, Carnegie Medicine, Stockholm, Sweden) to which a second cannula was glued by two component epoxy resin under microscopic and micromanipulator control. The second cannula served as guide for a concentric bipolar stimulation electrode (for details see Section 2.1) with 250 μm outer diameter and 30 mm total length.

The coordinates for implantation of the double guide cannula were: AP, −0.26 mm; ML, −3.0 mm relative to bregma; DV, −3.0 mm from the dural surface (Paxinos and Watson, 1988). Animals were allowed to recover from surgery for at least 5 days, before microdialysis experiments were performed.

On the day of the experiment, a microdialysis membrane (CMA/11, cut-off of 6 kDa, outer diameter 240 μm and membrane length 4 mm, Carnegie Medicine, Stockholm, Sweden) and a stimulation electrode were inserted into the guide cannula, under the condition of CO_2 and isoflurane anaesthesia to prevent animals from distress or pain. The location for the microdialysis probe was medial and for the stimulation electrode the lateral guide. The microdialysis probe and the stimulation electrode were lowered via guide cannula into the right caudate nucleus at the same depth with a minimal distance (<0.2 mm) between the microdialysis membrane and the stimulation electrode. All in vivo experiments were performed between 10:00 and 14:00 h.

The caudate nucleus was perfused with artificial cerebrospinal fluid (aCSF) consisting of (in mM): 125 NaCl, 5 KCl, 2 ($CaCl_2 \cdot 2H_2O$), 1.14 ($MgSO_4 \cdot 7H_2O$), 1.29 KH_2PO_4, 25 $NaHCO_3$, 0.1 ascorbic acid; pH 7.4 (Butcher et al., 1988; Thümen et al., 2002). A perfusion rate of 1.2 μl/min was selected. After a stabilization period of 2 h 15 consecutive dialysis samples of each 20 min sampling period (24 μl) were collected in 5 μl 3.3% perchloric acid, and stored at −22 °C until analysis. GABA and glutamate were analysed by means of HPLC with electrochemical detection after pre-column derivatization.

2.1. Electrical stimulation and animal groups

Sixty minutes after stabilization period, electrical stimulations were applied twice for a duration of 30 min each, from 60 to 90 min and from 150 to 180 min. At 240 min experiments were finished. Modified platinum–iridium (Pt/Ir) electrodes (CBCBG30, FHC Inc., Maine) were employed; they were bipolar, with the cathode in the center (diameter 75 μm), concentrically surrounded by the anode (Harnack et al., 2004). The total area of the smaller inner contact was 0.0062 mm^2. The conditions of electrical high frequency stimulation (HFS) were as followed: monopolar positive rectangular pulses of 124 Hz (Isostim A320D stimulator, WPI, Berlin, Germany), duration 60 μs. Constant current was 0.1 mA in group 1 ($n = 3$, awake) or 0.5 mA in group 2 ($n = 10$, awake) and in group 4 ($n = 5$, anaesthesized by sodium pentobarbital). The control group (group 3, awake) consisted of three rats with implantation as described above, however, without electrical stimulation. Rats were randomly allocated to the four groups. Animals of groups 1–3 were awake and active during sampling period. Animals of group 4 received HFS with a 0.5 mA current under sodium pentobarbital anaesthesia. All stimulation was controlled on-line by an oscilloscope.

2.2. HPLC

After precolumn derivatization with *o*-phthaldialdehyde and sodium sulphite for 10 min, GABA and glutamate values were measured using HPLC with electrochemical detection (Rowley et al., 1995; Smith and Sharp, 1994). The HPLC system consisted of a C18 column (Eurospher 100, 5 μm, column size 250 mm × 4 mm) and a precolumn (30 mm × 4 mm). The isocratic mobile phase (0.1 M sodium phosphate buffer, pH 4.5, containing 0.5 mM EDTA and 25% methanol) was previously degassed by helium and pumped at a flow rate of 1.0 ml/min and temperature of 30 °C. The compounds were detected electrochemically using a glassy carbon electrode set at a potential of 900 mV relative to an Ag/AgCl reference electrode (Waters 460 electrochemical detector, Millipore Corporation, Eschborn/Ts., Germany). Mean GABA and glutamate values were expressed in μM ± standard deviation (S.D.). The existence of differences between groups was assessed with one-way analysis of variance (ANOVA) with post hoc analysis for pairwise comparisons as indicated.

2.3. Histology

To check the correct position of the microdialysis probe and stimulation electrode histological examinations of brains were performed. After completion of the experiments rats were killed; the brains were quickly removed and preserved in formalin. Brain slices embedded in paraffin (5 μm) were stained with HE or trichrome standard techniques and examined under a light microscope. In all cases the microdialysis probe was correctly positioned in the caudate nucleus. Significant damage to the neuronal tissue or edema could not be observed around the tip of the electrode. In one animal of the 0.5 mA group a marked intracerebral bleeding was found near the position of the stimulation electrode.

3. Results

The following results are data to freely moving rats (groups 1–3). As shown in Fig. 1 basal glutamate values ranged from 12.9 to 14.3 μM at 0 min. During dialysis time glutamate values

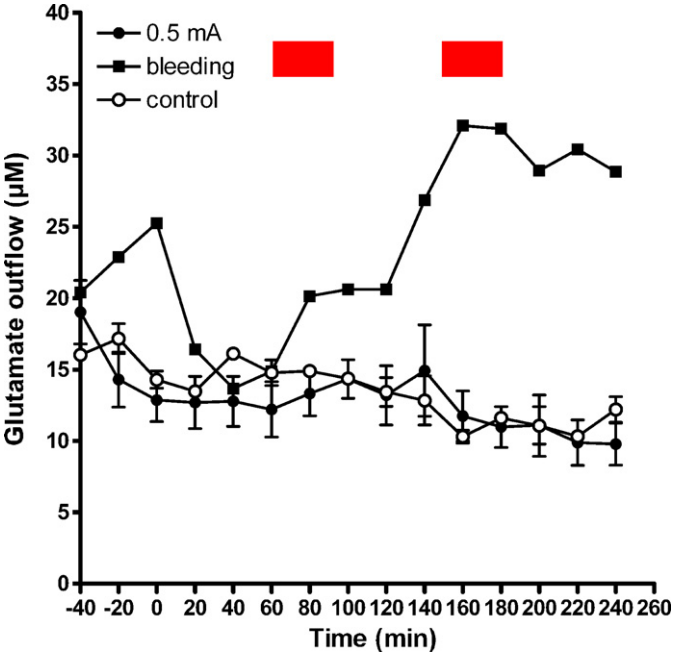

Fig. 1. Glutamate values were measured in the dialysates from the rat caudate nucleus by HPLC with electrochemical detection and expressed in μM ± S.D. HFS with 124 Hz was applied for 30 min each, from 60 to 90 min and from 150 to 180 min (bars). Control rats (◯, $n = 3$), 0.5 mA stimulation group (●, $n = 9$). In the rat suffering from a intracerebral bleeding, glutamate levels markedly increased (■).

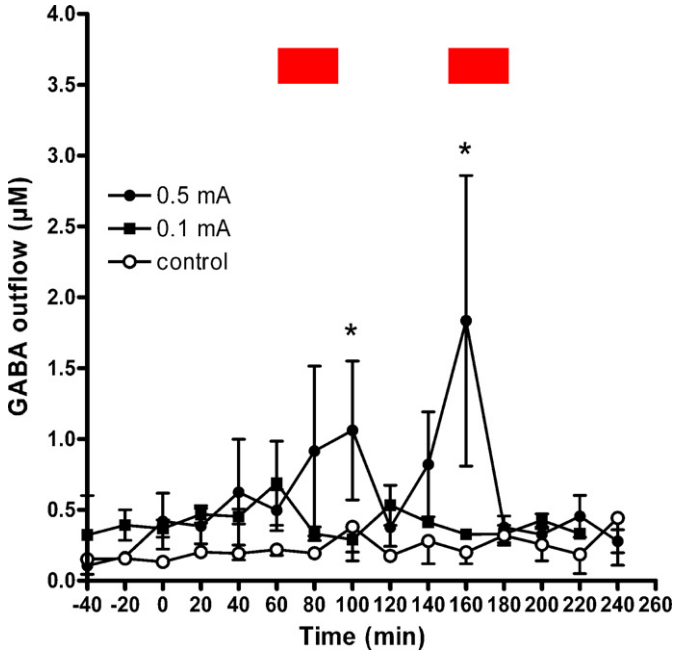

Fig. 2. GABA values were measured in the dialysates from the rat caudate nucleus by HPLC with electrochemical detection and expressed in μM ± S.D. HFS with 124 Hz was applied for 30 min each, from 60 to 90 min and from 150 to 180 min (bars). Control rats (◯, $n = 3$), 0.1 mA (■, $n = 3$) and 0.5 mA (●, $n = 9$) stimulation groups. * $p < 0.05$ significantly, when compared to controls.

slightly declined from 14.3 to 9.8 μM under control conditions. No significant differences of glutamate values were found between the 0.5 and 0.1 mA stimulation group and control animals. In the rat suffering from a significant intracerebral bleeding, glutamate levels markedly increased with a maximum of 32.1 μM at 160 min. This animal showed sluggish movements at the beginning but deep tiredness without reaction to noise and contact during the course until the end of the experiment.

Fig. 2 presents GABA values that were not significant different between groups at 0 min experimental time. GABA values ranged from 0.20 to 0.42 μM. The animal suffering from the bleeding was excluded from analysis of GABA levels.

In the case of 0.1 mA stimulation, no differences could be demonstrated between the stimulation and control groups. As the most outstanding result, GABA levels significantly increased to 1.06 or 1.83 μM, respectively, during or shortly after the stimulation period when a stimulation current of 0.5 mA was employed. However, it is noteworthy that during both stimulation periods GABA values showed a broad range and thus standard deviations of means were very high.

Additionally, animal behaviour was examined before, during, and after electrical stimulation. Except the animal with the bleeding, differences in the rats' behaviour could not be observed during the whole experiments.

Under full anaesthesia glutamate (0.52 μM) and GABA (0.027 μM) values were rather low in the dialysates and no HFS effect could be observed during the whole experiments with animals from group 4 (data not shown).

4. Discussion

The results described above demonstrated that simultaneous and collocated microdialysis and electrical high frequency stimulation (HFS) could be performed in the caudate nucleus of freely moving rats, and gave, therefore, directly evidence for local neuronal activity modified by HFS in vivo. When HFS was applied to freely moving rats during 30 min periods, extracellular GABA amounts increased in the caudate nucleus. No significant effects of HFS could be observed on glutamate outflow in all the experiments, which points to a specific effect of HFS on the GABAergic system. The GABA effect depended on the current used for HFS with GABA enhancement only at 0.5 mA. These findings are compatible with earlier in vitro experiments that showed HFS-induced GABA outflow from slices of the rat caudate nucleus (Li et al., 2004, 2006). Here, HFS seemed to interact with neuronal terminals of GABAergic medium-sized spiny interneurons. Medium-sized spiny neurons make up to 95% of the neostriatum and give rise to axons that leave the striatum and to profuse local axon collaterals that typically remain in the vicinity of the neuron of origin (Gerfen, 1988; Paxinos, 1995). Hence, medium spiny neurons have characteristics of both projection neurons and interneurons. According to Li et al. (2004) HFS in vitro only had an effect when neurons were pre-activated by the voltage-gated sodium channel opener veratridine; HFS in vitro was ineffective in resting GABAergic neurons of the caudate nucleus. Consistently, under our in vivo conditions HFS did not modulate GABA values in fully pentobarbital anaesthesized animals but in awake and active rats. Additionally, these results are partly compatible with a study from Beurrier et al. (2001)

who argued that HFS blocks the spontaneous activity of tonic and bursting STN neurons because these neurons are afferent to the GABAergic STN input (Obeso et al., 2000). Meissner et al. (2005) investigated the impact of HFS-STN on firing rate and oscillatory activity in the STN in MPTP-lesioned non-human primates. During HFS they found reduced firing rates and oscillatory activities of STN neurons. One explanation these authors proposed was that reduced neuronal activity may be the consequence of a transient GABAergic inhibition through excitation of presynaptic GABAergic axon terminals. This assumption is consistent with our findings, although caution is warranted in simply transferring the results from caudate nucleus to STN stimulation.

According to the literature in most of the studies HFS was performed in the STN. When microdialysis was employed simultaneously, in contrast to our experimental procedure the dialysis probes were not placed in the stimulated region but in other brain areas such as globus pallidus (GP), substantia nigra (SN), or caudate nucleus (Bruet et al., 2003; Meissner et al., 2004; Paul et al., 2000; Windels et al., 2005). For example, Windels et al. (2005) studied the effect of HFS-STN on extracellular glutamate and GABA levels in the GP and SNr of anaesthesized 6-hydroxydopamine-lesioned rats. In their experiments HFS-STN doubled the levels of extracellular GABA in the SNr of the hemiparkinsonian rats but did not affect glutamate levels. In a study of Paul et al. (2000) HFS-STN produced a delayed and intensity-dependent increase of extracellular dopamine metabolites but not of dopamine itself in the caudate nucleus of freely moving rats. These results are, however, more suitable to provide insights into functional network activity of the (lesioned) brain than into local biochemical HFS mechanisms.

It is noteworthy that in our experiments HFS-induced GABA outflow showed inter-individual differences. One explanation could be the complexity of the technical procedure, which probably led to distance variability between the microdialysis membrane and the stimulation electrode, although referring to histological examinations the distance was lower than 0.2 mm in all experiments. Another explanation may be the anatomical and physiological organisation of the caudate nucleus with main differences between rostral-caudal or medial-lateral parts (Paxinos, 1995). In our experiments charge ($Q = It$) per phase was 30 nC/phase and nearly equivalent in comparison to conditions of Harnack et al. (2004). The inner area of electrodes was, however, rather small to focus stimulation in the vicinity of the microdialysis membrane. Thus, charge density ($QD = ItA^{-1}$) was high with a maximum of 4.8 $\mu C/mm^2$/phase. Although electrode material plays a crucial role in the generation of tissue damage (Harnack et al., 2004; Riedy and Walter, 1996), especially when stainless-steel electrodes were used, this charge density is higher than the threshold given in the literature (McCreery et al., 1990) with values between 0.5 and 1 $\mu C/mm^2$/phase. Probably due to the short stimulation periods of 30 min each, tissue damage was not observed under our conditions.

Taken together, it is assumed that HFS has a specific effect on GABAergic neurons resulting in an enhancement of extracellular GABA levels in freely moving rats. There are significant differences of matrix organisation between the caudate nucleus and GPi/STN, and thus, caution is warranted in simply transferring the results to GPi or STN stimulation. Nevertheless, the present results give important insights into mechanisms by which HFS affects the neuronal system.

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