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ORIGINAL ARTICLE

Effects of GABA-transaminase inhibitor Vigabatrin on thermoregulation in rats

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Abstract Vigabatrin is a GABA derivative (gamma-vinyl GABA) which inhibits irreversibly the enzyme activity of GABA transaminase and thus increased indirectly brain GABA concentrations. We have used body temperature assay to examine the effects of Vigabatrin on thermoregulation in intact rats. In order to understand the mechanism of thermoregulatory action of Vigabatrin at cellular level, we have investigated its effect on individual warm-sensitive preoptic area/anterior hypothalamus (PO/AH) neurons in rat brain slice preparations. The results of the present study suggest that Vigabatrin produced dose-dependent hypothermia in rats and also increased temperature sensitivity of warm-sensitive PO/AH neurons.

Keywords GABA · Vigabatrin · Thermoregulation · PO/AH neurons · Rats

Introduction

Vigabatrin is an antiepileptic drug used for treatment of infantile spasms (West syndrome) and refractory complex partial seizures (Aicardi et al. 1996; Mumford and Cannon 1994). Vigabatrin is associated with a risk of developing visual field defects (Malmgren et al. 2001) that limited Vigabatrin to those patients with complex partial seizures refractory to other treatments (Waterhouse et al. 2009). Recent study showed the efficacy of Vigabatrin for the treatment of methamphetamine and/or cocaine addiction (Brodie et al. 2005) as well as anxiolytic effects of Vigabatrin in experimentally induced anxiety and panic disorder (Lang and de Angelis 2003; Zwanzger et al. 2001).

Vigabatrin is a structural analog of gamma-aminobutyric acid (gamma-vinyl GABA), and its S-enantiomer is pharmacologically active (Gram et al. 1989; Sheean et al. 1992). Vigabatrin increases brain GABA concentrations by irreversible inhibition of GABA transaminase (Gram et al. 1989; Mattson et al. 1994). Experimental studies observed that neuronal GABA transaminase is more sensitive than astrocytic GABA transaminase to S-Vigabatrin (Gram et al. 1989; Larsson et al. 1986).

GABA is a main inhibitory neurotransmitter in the CNS which is involved in the processes of thermoregulation. Animal studies reported that central or systemic administration of GABA and GABA agonists produced hypothermia (Yakimova and Ovcharov 1989; Zarrindast and Dibayan 1989). Preoptic area of the anterior hypothalamus (PO/AH) in the basal forebrain contains both temperature-sensitive (warm- and cold-sensitive) and temperature-insensitive neurons, which form synaptic network that controlled different thermoregulatory responses (Hori 1991). The presence of GABA has been found in various hypothalamic nuclei that suggests influence of GABAergic drugs on the PO/AH neurons (Gritti et al. 1993). GABA and GABA-receptor agonists inhibit the spontaneous activity (firing rate) of temperature-sensitive and temperature-insensitive PO/AH neurons, while the temperature sensitivity has been affected only in temperature-sensitive neurons (Yakimova et al. 1996).

The aim of the present study was to investigate the effects of Vigabatrin on core body temperature in conscious rats as well as on temperature sensitivity (TC) of

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warm-sensitive PO/AH neurons in rat hypothalamic slice preparations.

Materials and methods

Substances

S(+)-Vigabatrin was purchased from Sigma (Germany). The substance was administered centrally (intracerebroventricularly, i.c.v.). The doses used were based on literature data as well as on own previously made experiments.

Experimental animals

The experiments were carried out on male Wistar rats (weight range 200–250 g), which were divided into groups of 6–8 rats each. Rats were maintained on a standard 12 h light/dark cycle and allowed food and water ad libitum. Animal experiments were conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals.

In vivo experiments

Cannules implantation

Five days before central application of Vigabatrin, the rats were anesthetized with nembutal (30 mg/kg, i.p.) and ketamine (30 mg/kg, i.p.). Under aseptic conditions, stainless steel cannules were implanted by stereotaxic equipment into the right lateral ventricle using coordinates of Paxinos and Watson (1997): L 1.5; P 1.2; H 3.5. After implantation of cannules, rats were housed one per cage for a minimum of 5 days before experimental use. Vigabatrin (100 and 400 μg) was administered into the right lateral ventricle in a volume of 5 μl/rat using a Hamilton micro syringe. Dental acrylic cement was used to secure the cannules to the cranium. Skin was infiltrated with 1% lidocaine immediately after the suture in the order to eliminate postoperative suffering of the animals. The control animals were treated with saline (0.9% sodium chloride) in a volume of 5 μl/rat (i.c.v.). Following i.c.v. experiments, injection sites were verified with an injection of methylene blue (5 μl).

Body temperature measurements

All body temperature experiments started at 10 a.m. and were conducted at ambient temperature of 22 ± 1°C. Body temperature was measured with thermistor probes (TX8) and monitored on multichannel recorder Iso-Thermex 16 (Columbus Instruments, USA). The thermistor probes were lubricated and inserted rectally to a depth of 6 cm. Before drug administration, the initial temperature of the animals was determined. Body temperatures were recorded at 30th, 60th, 90th, 120th, 150th and 180th min following injection. The movements of the rats were slightly restricted as previously described Rosow et al. (1980).

In vitro experiments

In vitro experimental procedures were performed on hypothalamic slices (400 μm) of male Wistar rats as extracellular recordings of the neuronal activity (firing rate, imps/s) and temperature sensitivity (TC, imps/s/°C) in the conditions of perfusion with oxygenated artificial cerebrospinal fluid (ACSF) and superfusion with Vigabatrin (10 μM). The concentration of substance used in this investigation (Vigabatrin 10 μM) has showed the best effect in our previously made investigations.

Neuronal activity and slice temperature were recorded by conventional electrophysiological equipment, as previously described Schmid and Pierau (1993), and were stored on a personal computer using a 1401 interface (Cambridge Electronic Design, CED) and CED software Spike 2.

Recordings were carried out from spontaneously active neurons in the preoptic area of the anterior hypothalamus (PO/AH). Before application of the test substance, the temperature sensitivity of a neuron was determined using two sinusoidal temperature stimuli at intervals of 5 min. Superfusion of the test substance was started 5 min after the last control temperature stimulus, and test substance was applied for 5 min before the next temperature stimulus was performed. Superfusion returned to ACSF after this stimulus was completed, and further temperature stimuli were applied in anticipation of complete recovery. Only one neuron per slice was tested.

Data analysis and statistics

The results were calculated as delta (Δ) values (mean Δ value ± SEM). Transformed data were analyzed with two-way analysis of variance (ANOVA). For statistical significance a Student's t test was used. In all cases, values of $P < 0.05$ were considered to be statistically significant.

Temperature sensitivity was calculated by a computer program relating the discharge rate of the neuron (bin width = 5 s) to the actual temperature, and fitting either one linear or two piecewise regression lines through the data. The slope of the steepest regression line was used as the temperature coefficient (TC) of the unit (Vieht 1989). Warm-sensitive neurons were defined by a TC ≥ 0.6 imps/s/°C (Nakashima et al. 1987). The data are presented as mean ± SEM. For statistical evaluation, a paired t test was used.

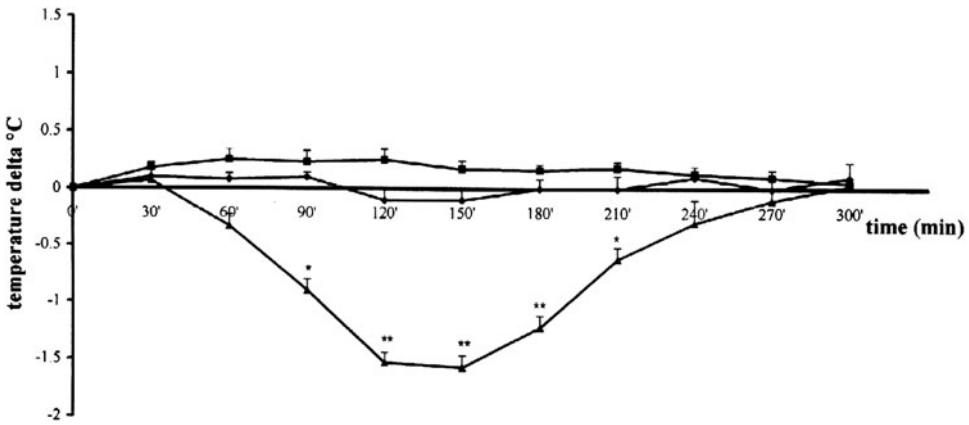

Fig. 1 Effects of intracerebroventricular application (i.c.v.) of Vigabatrin on body temperature in rats. Mean change (temperature delta °C) after administration of *filled squares* Vigabatrin 100 µg/5 µl/rat i.c.v., *filled triangles* Vigabatrin 400 µg/5 µl/rat i.c.v. and *filled diamonds* NaCl 5 µl/rat i.c.v. (control). Significant differences: $*P < 0.05$, $**P < 0.01$

Results

Effect of Vigabatrin on body temperature in rats

Vigabatrin administered i.c.v. in a dose 100 µg/5 µl/rat did not result in statistically significant change of core body temperature of rats (Fig. 1).

Intracerebroventricular administration of higher dose Vigabatrin (400 µg/5 µl/rat) induced a significant long-lasting hypothermia between 90th and 210th min after administration of the substance. The maximum response was observed at the 150th min after Vigabatrin application (Fig. 1). The control group receiving saline showed no change in the body temperature.

Effect of Vigabatrin on temperature sensitivity (TC) of warm-sensitive PO/AH neurons from the rat brain slice preparations

The superfusion of rat PO/AH neurons with Vigabatrin (10 µM) slightly decreased firing rate, but significantly increased temperature sensitivity (temperature coefficient, TC) in warm-sensitive PO/AH neurons ($P < 0.05$) (Figs. 2, 3).

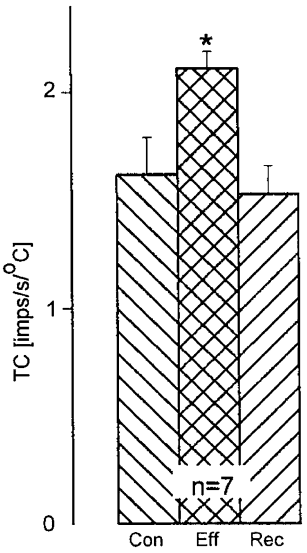

Fig. 2 Effect of Vigabatrin on temperature sensitivity (temperature coefficient, *TC*) of warm-sensitive rat PO/AH neurons. Average temperature coefficient (TC, imps/s/°C) of warm-sensitive rat PO/AH neurons before (control, *Con*), during (effect, *Eff*) and after (recovery, *Rec*) superfusion with Vigabatrin (10 µM); mean ± SEM; *n* number of neurons. Significant values: $*P < 0.05$

Discussion

Thermoregulation in mammals maintains the core temperature at a certain level. The thermoregulatory center located in the preoptic area/anterior hypothalamus (PO/AH) concerned mainly with the balance between heat gain and heat loss. Hypothalamic PO/AH neurons are important in regulation of body temperature. Gamma-aminobutyric acid (GABA) is involved in thermoregulation as well as other central neurotransmitters. The physiological balance between the heat production and heat loss in mammals is under the regulation of central neurotransmitters, including norepinephrine, dopamine, serotonin, acetylcholine and GABA.

In the present study we have examined the effects of Vigabatrin on body temperature in conscious rats and temperature sensitivity of warm-sensitive PO/AH neurons, treated with Vigabatrin in rat hypothalamic slice preparations. We have found that GABA-transaminase inhibitor Vigabatrin induces a dose-dependent decrease in core body temperature of rats and significantly increases temperature sensitivity (TC) of warm-sensitive PO/AH neurons in rat brain slice preparations.

As main central inhibitory neurotransmitter in mammals, GABA is involved in complex process of thermoregulation. Many experimental studies observed hypothermia after central or systemic administration of both direct- or

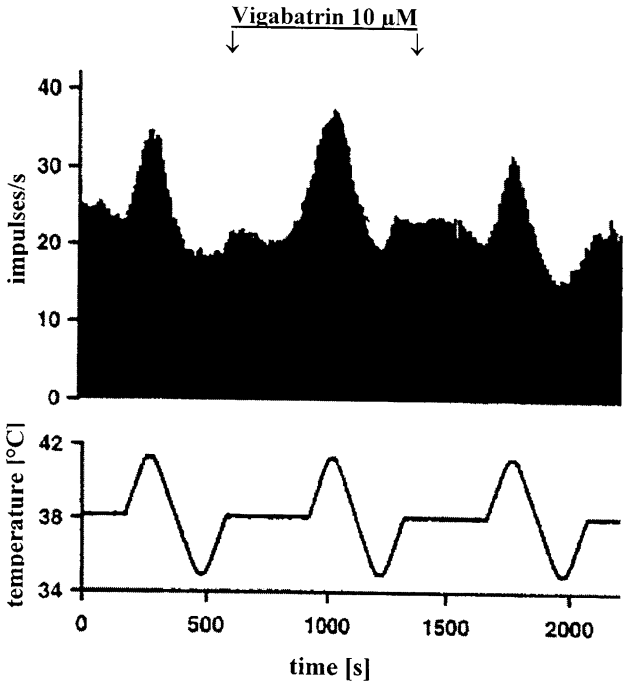

Fig. 3 Effect of GABA-transaminase inhibitor Vigabatrin on a warm-sensitive PO/AH neuron. Experimental protocol of neuronal activity (impulses/s) and temperature recorded close to the slice [temperature (°C)]. Calculated temperature coefficient (TC) before the superfusion (control) is 3.15 imps/s/°C. The superfusion of Vigabatrin (10 µM) increased temperature sensitivity (TC) of the warm-sensitive PO/AH neuron to 3.87 imps/s/°C (effect). Recovery of the TC (3.08 imps/s/°C) was observed after wash out

indirect-acting GABAergic drugs. These studies suggest that hypothermic reaction induced by GABAergic drugs, which act as direct GABA agonists, is mediated by activation of $GABA_A$ or $GABA_B$ receptors (Zarrindast and Dibayan 1989; Queva et al. 2003). The $GABA_A$ alfa-1 receptor subunit was reported to be essential for basal body temperature regulation (Vinkers et al. 2009). Animal and clinical studies demonstrated hypothermia induced by indirect GABA-acting drug Sodium Valproate (Nikolov and Yakimova 2008; Swaider et al. 2004; Nagarajan et al. 2001; Zachariah et al. 2000). The present study described significant long-lasting hypothermia, induced by intracerebroventricular administration of GABA transaminase inhibitor Vigabatrin. Inhibition of GABA degradation is the indirect mechanism which mediates decrease in body temperature. According to Gordon (1983), Vigabatrin-induced hypothermic reaction observed in this study can be classified as unresisted or regulated hypothermia.

It has been shown that about 30% of the spontaneously firing neurons in the PO/AH are warm-sensitive (Boulant 2001). Electrophysiological studies reported that substances, which induce hypothermia, increase temperature sensitivity of warm-sensitive rat PO/AH neurons (Pierau et al. 1998). In the present study, the superfusion of Vigabatrin on rat hypothalamic slices caused significant increase in temperature sensitivity of warm-sensitive PO/AH neurons.

The results from our study suggest that indirect GABA-acting drug Vigabatrin causes a dose-dependent hypothermia in rats by affecting the warm-sensitive neurons in PO/AH. Our results are step of understanding the complicated mechanisms of action of GABA-acting substances on thermoregulation.

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