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A microdialysis study on the mechanism of action of gabapentin

Wia Timmerman [a,*], Marielle Bouma [a], Jan B. De Vries [a], Michael Davis [b], Ben H.C. Westerink [a]

[a] *Department of Medicinal Chemistry, University Center for Pharmacy, Ant. Deusinglaan 1, 9713 AV Groningen, The Netherlands*
[b] *Parke-Davis Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105, USA*

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Abstract

To gain insight into the mechanism of action of the anti-epileptic, gabapentin, the effects of gabapentin on the in vivo extracellular γ-aminobutyric acid (GABA) levels in the rat substantia nigra reticulata were studied using microdialysis. In order to investigate possible interference with different GABA-ergic compartments in the substantia nigra reticulata, we studied the effects of gabapentin under basal, K^+-, nipecotic acid- and glutamate-stimulated conditions. Intraperitoneally (i.p.) administered gabapentin, at a dose of 100 mg/kg, did not significantly affect extracellular GABA levels under any condition. Thus, our data do not support the involvement of nigral GABA release in the mechanism of action of the anti-epileptic gabapentin.

Keywords: Gabapentin; Substantia nigra reticulata; γ-Aminobutyric acid; Microdialysis

1. Introduction

Gabapentin (Neurontin®) is a well-tolerated anti-epileptic drug, which is effective as monotherapy and add-on therapy to existing drug regimens for the treatment of refractory partial epilepsy (e.g. UK Gabapentin study group, 1990; Beydoun et al., 1998). The mechanism of action of gabapentin is not fully understood, but appears different from other anti-epileptic agents (Taylor et al., 1998). γ-Aminobutyric acid (GABA) receptor antagonists often induce focal or generalized seizures, whereas drugs that elevate GABA levels in the brain are used to treat various forms of epilepsy (Gale, 1989). Based on the importance of GABA-ergic transmission for the mechanism of action of anti-convulsive agents in general and gabapentin's structural similarity to GABA, gabapentin's anti-epileptic activity is presumed to operate through an enhancement of GABA-ergic activity. Drugs that limit seizure propagation by elevating GABA in the substantia nigra reticulata have a broad spectrum of anticonvulsant activity (Gale, 1989). The substantia nigra reticulata is therefore viewed as a 'common denominator' for the control of seizures originating in various brain areas. Gabapentin was found to enhance GABA turnover in the substantia nigra reticulata (Löscher et al., 1991) and it inhibited the firing of substantia nigra reticulata cells (Bloms-Funke and Löscher, 1996). Effects on nigral GABA release have not yet been determined.

In vitro studies have shown that in the striatum (Fichter et al., 1996) and hippocampus (Honmou et al., 1995a,b) gabapentin potentiates the nipecotic acid-induced non-vesicular GABA release postulated to occur via reversed operation of the GABA uptake carrier. Interestingly, the loss of non-vesicular glutamate-stimulated GABA release in the hippocampus was suggested to underly the epileptic pathology in human brain (During et al., 1995). Consequently, it has been hypothesized that gabapentin possibly compensates for this pathological reduction in transport-mediated GABA release in epileptic brain by promoting non-vesicular GABA release (Taylor et al., 1998). Although this is an intriguing possibility, others found that although GABA-elevating drugs were able to increase GABA in compartments that were not directly related to synaptic transmission in the substantia nigra reticulata, only the nerve-terminal-associated GABA levels predicted the anticonvulsant potential of a GABA-elevating agent (Gale, 1989).

* Corresponding author. Tel.: +31-50-3633305; fax: +31-50-3636908.
E-mail address: wia@farm.rug.nl (W. Timmerman).

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In this study, we examined the effects of gabapentin on the in vivo extracellular GABA levels in the rat substantia nigra reticulata using microdialysis. To investigate possible interference with different GABA-ergic compartments in the substantia nigra reticulata, we studied the effects of gabapentin under various conditions, i.e. basal-, K^+-, nipecotic acid- and glutamate-stimulated conditions. Since basal extracellular GABA levels for the most part do not seem to reflect neuronal activity (Timmerman and Westerink, 1997), K^+-stimulation was used to release Ca^{2+}-dependent vesicular GABA release (Westerink and De Vries, 1989). Nipecotic acid- and glutamate-stimulated conditions were used based on studies mentioned above in which these conditions are postulated to stimulate GABA release via reversed uptake carriers (During et al., 1995).

2. Materials and methods

2.1. Animals and drugs

Male albino Wistar rats (250–350 g; C.D.L., Groningen, The Netherlands) were housed in plastic cages ($35 \times 35 \times 40$ cm) and had free access to food and water. Animal procedures were conducted in accordance with guidelines published in the NIH guide for the care and use of laboratory animals and all protocols were approved by the Groningen University Institutional Animal Care and Use Committee. Gabapentin (Neurontin®) was donated by Parke-Davis Pharmaceutical Research (Ann Arbor, MI) and intraperitoneally (i.p.) administered. Nipecotic acid and glutamate (Sigma, St. Louis, MO) were dissolved in the Ringer solution and infused via the microdialysis membrane.

2.2. Surgery and brain microdialysis

An ''I''-shaped cannula (inner diameter: 0.22 mm; outer diameter: 0.31 mm; AN 96, Hospal, Bologna, Italy) was implanted in the substantia nigra reticulata under chloralhydrate anaesthesia (400 mg/kg i.p.) with local application of 10% lidocaïne. Coordinates were A/P 3.3 from interaural, L/M 3.9 and V/D 8.5, inserted under an angle of 15° in the coronal plane. The exposed tip of the probe was 1.5 mm. The in vitro recovery of the membrane for GABA was 6% at a flow rate of 3 μl/min and 37°C.

The experiments were performed in conscious rats 24–48 h after surgery. The microdialysis probe was perfused with a Ringer solution (147 mmol/l NaCl, 4 mmol KCl, 1.2 mmol $CaCl_2$, 1.1 mmol/l $MgCl_2$) at a flow rate of 3 μl/min. GABA was quantified after precolumn derivatization by isocratic high-performance liquid chromatography (HPLC) separation in conjunction with fluorimetric detection. An automated on-line HPLC assay (Westerink and De Vries, 1989) was used with minor modifications. The outlet tube of the dialysis probe was connected via a ''T''-piece with the tubing containing the derivatization reagent (flow rate 1 μl/min). The mixture was directly led into a 50-μl load loop of an HPLC valve, in which the derivatization took place. The valve was held in the load position for 15 min and was switched automatically to the inject position for 15 s. A second loop (300 μl) was continuously filled with 90% (v/v) ethanol and directly after the GABA peak was recorded, the loop content was injected onto the column to quickly elute all subsequent peaks.

The HPLC system consisted of an LKB 2150 HPLC pump (Pharmacia, The Netherlands), an S3 ODS2 Spherisorb column with 3-μm particle size, Rheodyne valves (California, USA) and a fluorimeter (excitation, 340 nm; emission, 400 nm; Shimadzu RF-10A, Sweden). The brain was perfused using a CMA 102 microdialysis pump (Sweden). A mobile phase consisting of 0.05 mol/l Na_2HPO_4, 0.01 mmol/l Na_2EDTA, 0.6% (v/v) tetrahydrofuran, and 45% (v/v) methanol (pH 5.95 with phosphoric acid) was used. The derivatization reagent was prepared as follows: 5 mg *o*-phthaldialdehyde was dissolved in 50 μl methanol and added to 5 ml 0.5 mol/l $NaHCO_3$ (pH adjusted to 9.5 with a sodium hydroxide solution) containing 15 μl 2-mercaptoethanol. The reagent was prepared fresh every day.

2.3. Histology

Upon completion of the experiments, each rat was deeply anaesthesized with chloral hydrate and perfused through the ascending aorta with a solution of 4% paraformaldehyde solution. The brain was removed and frozen and slices of 16 μm were taken to identify the placements of the probes.

2.4. Statistics

A two-way analysis of variance (ANOVA) with repeated measures was used followed by the Newman–Keuls multiple comparison test to compare the effect of gabapentin vs. saline injections under basal conditions and to compare K^+, nipecotic acid and glutamate infusions with and without gabapentin injections, respectively.

3. Results

3.1. Basal levels

The average of four pre-treatment samples was taken as 100% baseline and all post-treatment samples were expressed relative to the basal values, without correcting for microdialysis probe recovery of GABA. The average dialysate concentration ($\pm$S.E.M.) for GABA was 69 ± 49 fmol/min ($n = 33$).

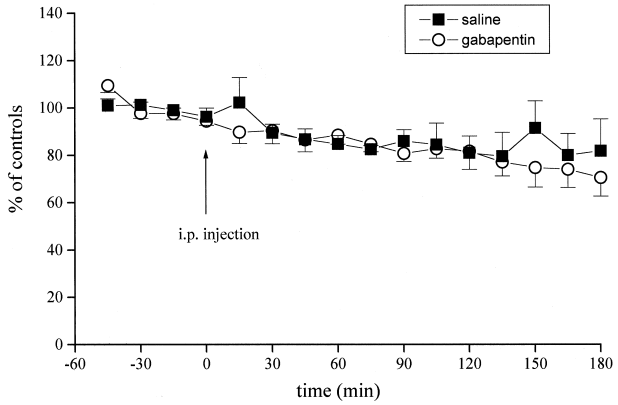

Fig. 1. Effects of i.p. injections of saline (-■-) ($n = 4$) and gabapentin (-○-) (100 mg/kg; $n = 4$) on extracellular GABA concentrations in the substantia nigra reticulata. The arrow indicates the moment at which gabapentin was injected. Data are given as percentage of control values ± S.E.M.

3.2. Effect of gabapentin on basal GABA levels

After i.p. injection of either saline or gabapentin (100 mg/kg), a slight decrease in the levels of GABA in the substantia nigra reticulata was seen (Fig. 1), though the pre-drug basal levels showed a tendency to decrease before the injections were administered. There was no significant difference between the GABA levels after saline and gabapentin injections.

3.3. Effects of gabapentin on stimulated GABA levels

Infusion of K^+ (60 mM) in the Ringer solution into the substantia nigra reticulata caused an immediate increase in GABA levels (to 400% of basal values), which slightly declined in time (Fig. 2). Three hours after the start of the K^+ infusion, the output of GABA was about 250% of basal values. I.p. injection of gabapentin (100 mg/kg) 1 h after the start of the K^+ infusion did not significantly affect GABA levels for the subsequent 3 h. Nipecotic acid (500 μM) infusion into the substantia nigra reticulata induced an immediate increase in GABA levels. During the infusion period, the GABA levels rose to and remained approximately stable at 300% of basal values (Fig. 3). In a separate group, following administration of gabapentin (100 mg/kg i.p.) 1 h after the start of the nipecotic acid infusion, the GABA output was approximately stable for 3 h at 350–400% of basal values. Although initial increases in GABA levels were deviant, there was no significant difference between the two curves during the entire experi-

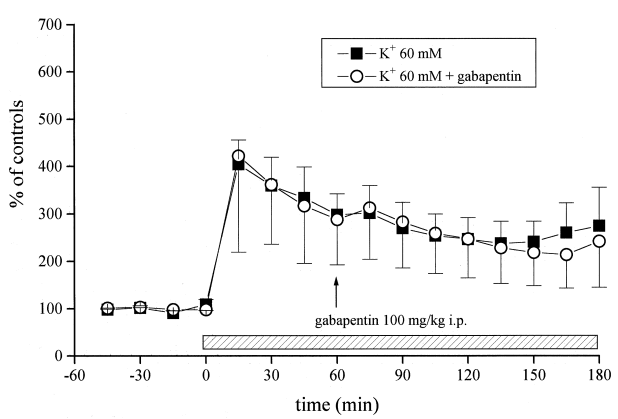

Fig. 2. Effects of K^+ infusion (60 mM) on the extracellular levels of GABA in the substantia nigra reticulata (-■-) ($n = 4$). Effects of gabapentin (100 mg/kg i.p.) on extracellular levels of GABA in the substantia nigra reticulata, which were stimulated by K^+ infusion (60 mM) (-○-) ($n = 5$). The bar indicates the period of high K^+ infusion, and the arrow the moment at which gabapentin was injected. Data are given as percentage of control values ± S.E.M.

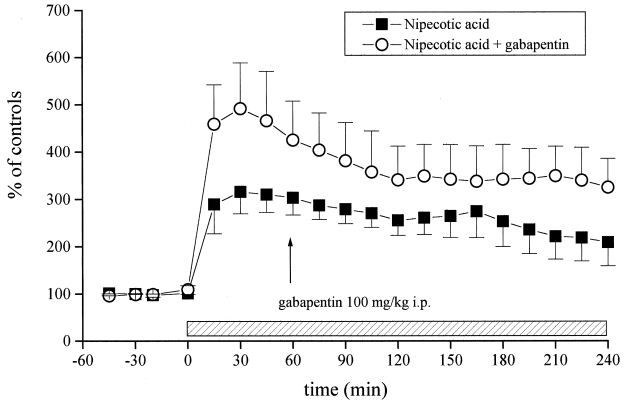

Fig. 3. Effects of nipecotic acid infusion (500 μM) on the extracellular levels of GABA in the substantia nigra reticulata (-■-) ($n = 4$). Effects of gabapentin (100 mg/kg i.p.) on extracellular levels of GABA in the substantia nigra reticulata during nipecotic acid infusion (500 μM) (-○-) ($n = 4$). The bar indicates the period of nipecotic acid infusion, and the arrow the moment at which gabapentin was injected. Data are given as percentage of control values ± S.E.M.

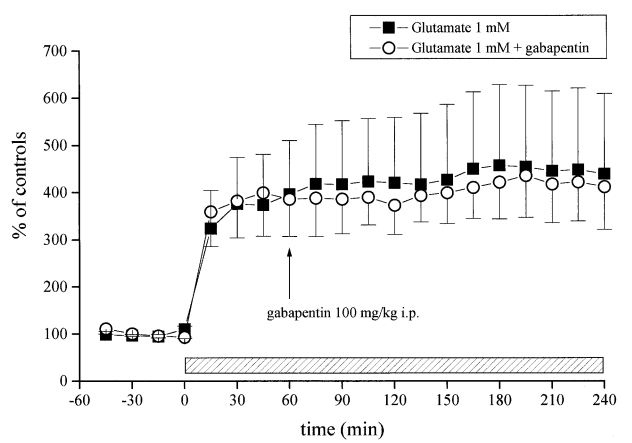

Fig. 4. Effects of high glutamate concentration in the Ringer solution (1 mM) on the extracellular levels of GABA in the substantia nigra reticulata (-■-) ($n = 4$). Effects of gabapentin (100 mg/kg i.p.) on extracellular levels of GABA in the substantia nigra reticulata during high glutamate concentrations in the Ringer solution (1 mM) (-○-) ($n = 4$). The bar indicates the infusion period of high glutamate concentrations, and the arrow the moment at which gabapentin was injected. Data are given as percentage of control values ± S.E.M.

mental period. Glutamate infusion (1 mM) into the substantia nigra reticulata induced an increase in GABA levels to approximately 400% of basal values, which remained approximately stable over the experimental period (Fig. 4). Gabapentin injection (100 mg/kg i.p.) 1 h after the start of the glutamate infusion showed no significant effect on GABA levels for the subsequent 3 h.

4. Discussion

In this study, the anti-epileptic drug, gabapentin had no significant effect on basal or stimulated GABA levels in the substantia nigra reticulata. Gabapentin was used in an anti-convulsive dose based on the fact that an ED_{50} value of 30–100 mg/kg (i.p.) for the anticonvulsant effect of gabapentin was found using hippocampal-kindled rats (Goa and Sorkin, 1993). The maximal anticonvulsant action has been shown to be reached not before 2 h after administration and lags behind peak drug concentrations in brain tissue and microdialysates (Welty et al., 1993). Still, gabapentin did not affect extracellular GABA levels in the substantia nigra reticulata during 2–3 h after administration under the various conditions. Given the fact that local applications of GABA receptor agonists and GABA elevating agents into the substantia nigra reticulata have been shown to suppress seizures in different models of epilepsy (Gale, 1989) and the findings that gabapentin increased GABA turnover in the substantia nigra reticulata (Löscher et al., 1991) and inhibited the firing rate of substantia nigra reticulata cells (Bloms-Funke and Löscher, 1996), gabapentin was expected to stimulate the release of nigral GABA.

At first glance, the lack of effect of gabapentin under basal conditions suggests that the compound does not affect extracellular GABA levels in the substantia nigra reticulata under non-stimulated conditions. This would be in line with the hypothesis that the activity of gabapentin may be limited to hyperactive neuronal circuits (Fichter et al., 1996). On the other hand, it cannot be excluded that GABA release was indeed increased, but this increase was not reflected in the overall extracellular GABA levels. Two explanations are considered here. Firstly, the substantia nigra reticulata contains various sources of GABA-ergic inputs. Since collaterals of substantia nigra reticulata neurons substantially contribute to the release of GABA in the substantia nigra reticulata (Lantin le Boulch et al., 1991), the inhibition of these substantia nigra reticulata cells may have counteracted possible increases in GABA release upon gabapentin. Still, as we studied effects of gabapentin under four different conditions (basal, high K^+, glutamate and nipecotic acid), this explanation seems less likely. Secondly, potential effects of gabapentin on the neuronal compartment are not likely to be detected under basal conditions, because of methodological considerations. In the extracellular space, different compartments of GABA have been postulated (e.g. Gale, 1989; Timmerman and Westerink, 1997). Under non-stimulated conditions, neuronal exocytotic GABA release is for the most part not reflected in the extracellular GABA levels measured by microdialysis (Timmerman and Westerink, 1997). A similar explanation might be given for the lack of effect on basal GABA levels in the substantia nigra reticulata of the anti-epileptic, valproic acid measured by microdialysis (Sayin et al., 1995), despite the fact that valproic acid was found to affect the neuronal compartment of GABA in the substantia nigra reticulata using other techniques (Gale, 1989).

K^+-stimulated GABA release is Ca^{2+}-dependent (Westerink and De Vries, 1989) and therefore, most likely reflects exocytotically released GABA in contrast to basal levels that are for the most part tetrodotoxin- and Ca^{2+}-independent (Timmerman and Westerink, 1997). If nerve-terminal-associated GABA release is crucial for seizure control (Gale, 1989), the K^+-stimulated increase in GABA levels was expected to be affected by gabapentin. Still, gabapentin did not modulate the elevated GABA levels, suggesting that it does not interfere with stimulated, exocytotically released GABA in the substantia nigra reticulata. Although K^+-stimulation is often used to study neuronal GABA release, it has to be kept in mind that high K^+ concentrations affect a variety of amino acids and transmitter systems in the brain (Westerink et al., 1987), which may have interfered with potential effects of gabapentin.

The anticonvulsant effect of gabapentin has been hypothesized to involve the promotion of non-vesicular GABA release (Taylor et al., 1998). The latter type of GABA release was postulated to be reduced in the epileptic brain (During et al., 1995). In vitro studies showed that gabapentin enhanced the nipecotic acid-evoked [^{3}H]GABA release in striatal slices (Fichter et al., 1996) and potentiated the nipecotic acid-induced effects on the postsynaptic potential in hippocampal slices (Honmou et al., 1995a,b). The response of nipecotic acid in the hippocampus was also present after blockade of synaptic transmission by the use of a Ca^{2+}-free solution, which pointed to the GABA releasing properties of nipecotic acid and not its reuptake inhibiting properties. The authors suggested that nipecotic acid induced this GABA release via reversal of the uptake carriers. Indeed, using microdialysis, nipecotic acid-induced increases in extracellular GABA levels are found to be for the most part tetrodotoxin- and Ca^{2+}-independent (Westerink and de Vries, 1989; Campbell et al., 1993), which suggests that they originate from non-exocytotic release. Also, glutamate-induced GABA release has been hypothesized to be of non-exocytotic origin (During et al., 1995). The present report evaluated the involvement of non-vesicular release in the mechanism of action of gabapentin, by studying the effects of gabapentin during nipecotic acid- and glutamate-stimulated conditions. The absence of effects of gabapentin during stimulation by nipecotic acid or glutamate does not support the notion

that non-vesicular release is involved in the mechanism of action of gabapentin under the chosen conditions. Whether or not non-vesicular GABA release could reflect release mediated via reversal of uptake carriers is presently unclear, since the concept of functional reversed carrier-mediated release of GABA in vivo is still under debate (reviewed in Timmerman and Westerink, 1997). The GABA carriers have been shown to be driven to release GABA when ionic gradients are perturbed in a specific manner, but whether these pronounced ion shifts occur under physiological conditions is not unequivocally determined. Even neuronal depolarizations as a result of seizure activity are questioned to be sufficiently strong to reverse GABA transporters (Meldrum, 1995).

Our data do not give evidence for the involvement of nigral GABA release in the mechanism of action of the anti-epileptic gabapentin. Gabapentin did not affect basal extracellular GABA levels, nor the K^+-, glutamate- and nipecotic acid-induced increases in GABA levels in the substantia nigra reticulata.

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