ELSEVIER

European Journal of Pharmacology 316 (1996) 211–218

ejp

The anticonvulsant gabapentin decreases firing rates of substantia nigra pars reticulata neurons

Petra Bloms-Funke, Wolfgang Löscher *

Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, Bünteweg 17, 30559 Hannover, Germany

Received 2 May 1996; revised 12 August 1996; accepted 20 August 1996

Abstract

Gabapentin is a novel anti-epileptic drug which enhances GABA (γ-aminobutyric acid) turnover in certain brain regions, including substantia nigra. However, the functional consequences of GABA turnover increases in response to gabapentin and their potential involvement in the anticonvulsant action of this drug are not known. In the present study, we examined the effects of gabapentin on the extracellular, single unit activity of nondopaminergic (presumably GABAergic) neurons of the substantia nigra pars reticulata in rats. During the recordings, the animals were infused with the narcotic opioid analgesic fentanyl, associated with a skeletal muscle relaxant and artificial ventilation. The spontaneous firing of substantia nigra pars reticulata neurons was determined up to about 2 h after i.v. or i.p. administration of gabapentin at doses of 15–30 mg/kg. After both routes of administration, gabapentin markedly reduced neuronal firing when administered at a dose of 20–30 mg/kg, while 15 mg/kg were ineffective in this regard. The suppressive effect of gabapentin was rapid in onset (2 min after i.v. and about 20 min after i.p. injection), reached peak values of about 70% below predrug baseline after about 45–60 min, and remained at this level for at least 2 h. Vehicle administration had no effect on substantia nigra pars reticulata neurons. The ability of gabapentin to alter substantia nigra pars reticulata firing does correlate with its known ability to increase nigral GABA turnover. Since a substantial body of evidence suggests that the substantia nigra pars reticulata is a critical site at which decrease of neuronal firing by potentiation of GABAergic influences results in protection against various seizure types, the suppressive effect of gabapentin on substantia nigra pars reticulata activity may contribute to the anticonvulsant action of this drug.

Keywords: GABA (γ-aminobutyric acid); Anti-epileptic drugs; Epilepsy; Midbrain

1. Introduction

The anti-epileptic drug gabapentin (1-[aminomethyl]cyclohexaneacetic acid) was designed in the late 1970s as a GABA (γ-aminobutyric acid)-related amino acid which, unlike GABA, passes the blood–brain barrier but retains as much as possible of the chemical and physical properties of the inhibitory neurotransmitter (Bartoszyk et al., 1986). However, numerous subsequent studies indicated that gabapentin is inactive at $GABA_A$ and $GABA_B$ receptors, does not interact with GABA uptake and exerts only weak effects on the GABA degrading enzyme GABA aminotransferase (Bartoszyk et al., 1986; Taylor, 1995). For several years it was therefore thought that gabapentin, despite its name, does not interact with the GABA system, and its mechanism of action was unknown (Bartoszyk et al., 1986). More recently, we found that gabapentin increases GABA turnover, i.e., the apparent rate of synthesis of GABA, in several brain regions, suggesting that increased GABA metabolism may underlie gabapentin's anticonvulsant action (Löscher et al., 1991). Interestingly, similar to the anti-epileptic drug valproate (Löscher, 1989), there was no uniform increase of GABA turnover across brain regions, but the effect of gabapentin appeared to occur in only some discrete regions, including substantia nigra (Löscher et al., 1991). The latter brain structure has been proposed to represent a 'key region' in a preferred propagation pathway of many types of experimental seizures, and it has been suggested that drugs that limit seizure propagation through these common pathways have a broad spectrum of anticonvulsant activity (Gale, 1988, 1992; Löscher and Ebert, 1996). Indeed, reduction of firing rate of GABAergic output neurons in substantia nigra pars reticulata by microinjection of GABAmimetic drugs into substantia nigra pars

* Corresponding author. Tel.: (49-511) 856-8721; Fax: (49-511) 953-8581.

PII S0014-2999(96)00682-6

reticulata has been demonstrated to be a very effective means of blocking a variety of seizure types (Gale, 1988, 1992; Löscher and Ebert, 1996). Interestingly, systemic administration of the standard anti-epileptics valproate, phenobarbital, diazepam, and clonazepam was demonstrated to inhibit firing of substantia nigra pars reticulata neurons, an effect which could be critically involved in the broad anticonvulsant action of these drugs, which are known to interact with GABAergic transmission (Waszczak et al., 1986; Löscher et al., 1995; Rohlfs et al., 1996). In the present study, we examined whether gabapentin affects the extracellular, single unit activity of non-dopaminergic substantia nigra pars reticulata neurons in rats.

2. Materials and methods

2.1. Animals

If not otherwise indicated, female Wistar rats (Harlan Winkelmann, Borchen) weighing 220–260 g (age about 4 months) were used. The animals were purchased from the breeder at a body weight of about 200 g. Following arrival in the animal colony, the rats were kept under controlled environmental conditions (ambient temperature 24–25°C, humidity 50–60%, 12/12 h light/dark cycle, light on at 7.00 a.m.) for at least 1 week before being used in the experiments. Standard laboratory chow (Altromin 1324 standard diet) and tap water were allowed ad libitum. In some experiments, female Wistar rats ($n = 3$) of higher age (about one year) were used to examine if gabapentin's effects changed with age. Furthermore, some experiments were done with female Sprague–Dawley rats ($n = 3$) to examine the influence of strain. A total of 43 rats were used for the present experiments.

2.2. Neurophysiological studies

The spontaneous firing pattern of single non-dopaminergic (presumably GABAergic) neurons of substantia nigra pars reticulata was examined by intranigral extracellular single-unit recordings using standard techniques, as described in detail recently (Löscher et al., 1995; Rohlfs et al., 1996). As in these previous studies of our group, female rats were used in the present investigation. The rats were anesthetized with the short acting barbiturate methohexital (60–70 mg/kg), combined with an i.p. injection of 50 μg/kg of the 'narcotic' opioid analgesic fentanyl, to achieve surgical anesthesia for preparation of the animals. Additional injections of 20 mg/kg methohexital i.p. or (after cannulation of the vena femoralis) 12 mg/kg i.v. were given as needed until all surgical procedures were finished. The rats were vagotomised bilaterally in order to prevent marked salivation (and the associated risk of saliva aspiration) in response to methohexital and vagal adverse effects of fentanyl. Bolus doses of methohexital and fentanyl were based on preliminary experiments in rats, showing that both drugs interacted in a synergistic fashion so that anesthetic doses of methohexital could be reduced by about 50% (Löscher et al., 1995). After cannulation of a vena femoralis, fentanyl and the muscle relaxant gallamine were infused throughout the experiment at a rate of 100 μg/kg per h (fentanyl) and 20 mg/kg per h (gallamine), respectively. A bolus dose of 20 mg/kg gallamine was given i.v. at onset of gallamine infusion. After tracheotomy and intubation, the rats were artificially ventilated with room air at a rate of 75/min with a tidal volume (2–3.5 ml) to suppress spontaneous breathing and maintain an expired CO_2 level of 2.4–2.6%, as measured by a CO_2 gas analyser. To assure stability of anesthesia, heart rate, arterial blood pressure (measured via the arteria femoralis), and body temperature were continuously monitored during the experiment. Furthermore, short foot-pinches were applied by a hemostat to test depth of anesthesia. The infusion rate of fentanyl was reduced during the experiment if too marked depression of heart frequency or blood pressure developed, or was enhanced if a defence reflex or heart rate increase to foot-pinch occurred. Body temperature was recorded by a rectal probe and maintained at 36–38°C by a heat-pad. A single barrel glass microelectrode, filled with 3% horseradish peroxidase in Tris-buffered saline, was lowered through a small burr hole in the skull to the level of just above the left substantia nigra pars reticulata. Stereotactic coordinates (measured from bregma according to the coordinates of Paxinos and Watson (1986)) were as follows: anterior–posterior (AP) −5.5, lateral (L) +2.2, ventral (V) −6.0. The electrode was than slowly lowered under continuous recording of extracellular neuronal signals until a non-dopaminergic substantia nigra pars reticulata neuron could be identified. Standard techniques for amplifying, discriminating and counting extracellular single-unit action potentials were used. Neurons were identified as nondopaminergic substantia nigra pars reticulata neurons during the recording period on the basis of their location and electrophysiological characteristics. Specifically, nondopaminergic, presumably GABAergic substantia nigra pars reticulata neurons were encountered typically after passing through a more dorsal zone of substantia nigra pars compacta dopaminergic neurons, whose electrophysiological characteristics are easily recognized and differentiated from nondopaminergic neurons (Bunney et al., 1973; Guyenet and Aghajanian, 1978; Waszczak et al., 1986). Non-dopaminergic substantia nigra pars reticulata neurons were located beneath the dopamine neurons, and they exhibited the following extracellular characteristics, as described previously (Guyenet and Aghajanian, 1978; Waszczak et al., 1986): smooth, sharp, biphasic action potentials with a duration of 0.5–1.2 ms (versus 1.5–4.5 ms in case of dopaminergic neurons) and firing rates of 10 to 40 action potentials/s (versus 3–6 Hz in case of dopaminergic

neurons). If no such neuron with stable firing rate could be identified, the electrode was removed and lowered again by changing either the AP (from −4.8 to −5.8 mm) or L (from 1.8 to 2.6 mm) coordinates, respectively. In animals in which no nondopaminergic neuron in the left substantia nigra pars reticulata could be recorded, the right substantia nigra pars reticulata was used for neuronal recording. After identifying a nondopaminergic substantia nigra pars reticulata neuron by the characteristics described above, and following stabilisation of this neuron, the spontaneous firing rate was monitored and averaged over a period of 10 min (baseline period). Thereafter, gabapentin, 15–30 mg/kg, was injected i.v. or i.p., and firing rate was recorded for a subsequent period of at least 50 min. The dose range of gabapentin tested was selected on the basis of its anticonvulsant action against maximal electroshock seizures in female Wistar rats, in which significant anticonvulsant effects were obtained after 20–30 mg/kg (W. Löscher and D. Hönack; unpublished experiments). In separate control experiments, vehicle (saline) was injected instead of drug. Only one neuron was studied per rat. Recording of drug or vehicle effects on substantia nigra pars reticulata neurons was started about 70 min after the last injection of methohexital, assuming that the barbiturate had been largely eliminated from the brain at this time. During recording, neuronal firing rates were averaged over 10 s periods by the MRATE program (CED, Cambridge Electronic Design, Cambridge). For graphic illustration of baseline values and post-drug values as a function of time after drug administration, mean values for subsequent 2 min periods were calculated.

At the end of the recording period, the location of the electrode tip was marked by iontophoretical ejection of a small amount of horseradish peroxidase (Simons and Land, 1987). For this purpose, a pulsed cathodal current (5 s on, 5 s off) of 1 μA was applied for 2 min. The electrode was then withdrawn, and the respective anesthetized animal was perfused transcardially with phosphate-buffered saline followed by 4% phosphate-buffered paraformaldehyde (pH 7.3). The brain was then removed and stored in 30% sucrose overnight at 4°C. Subsequently, coronal sections (60 μm) were cut on a freezing microtome, and sections were incubated with diaminobenzidine in presence of Ni^{2+} ions (Adams, 1981) to stain the horse radish peroxidase spots. After mounting on glass slides, the sections were counter-stained with neutral red. Location of the black spot within the substantia nigra pars reticulata verified correct placement of the recording electrode. Only rats with electrode location in substantia nigra pars reticulata were used for further evaluation of data.

2.3. Drugs

Gabapentin was kindly provided by Parke-Davis (Ann Arbor, MI, USA). The drug was freshly dissolved in saline before each experiment and was injected in a volume of 2 ml/kg. Controls received the same volume of saline.

2.4. Statistics

Statistical significance of differences between drug treated and control groups was calculated by the Mann-Whitney U-test. Significance of differences within groups before and after drug injection was evaluated by the Friedman test, followed by the Wilcoxon rank test for paired replicates. Nonparametric statistics were used because a distribution analysis did not indicate a normal distribution of data.

3. Results

3.1. Location and baseline activity of recorded substantia nigra pars reticulata neurons

From a total of 43 rats used for extracellular recording of substantia nigra pars reticulata neurons in this study, 26 fulfilled all inclusion criteria (recording from a single nondopaminergic substantia nigra pars reticulata neuron for at least 50 min, correct location of electrode) and could thus be used for final evaluation. Fig. 1 illustrates the within substantia nigra pars reticulata locations of these neurons. Baseline activity of these substantia nigra pars reticulata neurons in the 10 min period before vehicle or drug administration ranged between 13 and 42 action potentials per second; mean ± S.E. was 24 ± 2 action potentials per second. Foot-pinches had no effect on spontaneous activity of any of the non-dopaminergic substantia nigra pars reticulata neurons recorded at the locations shown in Fig. 1.

3.2. Effect of gabapentin on spontaneous activity of substantia nigra pars reticulata neurons

Following i.v. or i.p. administration of gabapentin (15 or 30 mg/kg), heart frequency slightly decreased from 417–510 (464 ± 9; $n = 13$) beats per min in the baseline period before drug injection to 360–490 (442 ± 11) beats per min at the end of the postdrug period. Blood pressure, which ranged between 80–130 (94 ± 6) mmHg and 85–130 (99 ± 4; $n = 13$) mmHg before and after drug application, respectively, was not significantly changed. I.v. and i.p. injections of saline did not induce any significant effects on blood pressure or heart rate ($n = 9$).

After i.v. injection of 30 mg/kg of gabapentin, firing rate of substantia nigra pars reticulata neurons rapidly decreased in all 6 animals of the respective group (Fig. 2A). Maximal depression was reached in all neurons within 30–40 min after drug application and there was no trend towards predrug control values over the average period of postdrug recording (Fig. 2A). In one rat, in which a substantia nigra pars reticulata neuron could be recorded

for more than 2 h after drug injection, substantia nigra pars reticulata firing was decreased over the whole postdrug period (Fig. 2B), demonstrating a long duration of this effect of gabapentin. The maximal depression of substantia nigra pars reticulata firing in response to gabapentin ranged between 65–80% (compared to predrug baseline). The respective group of 6 animals shown in Fig. 2A was composed half of younger and half of older Wistar rats (see Section 2), which showed no difference in the above described effects of gabapentin and, thus, were pooled. The reduction of substantia nigra pars reticulata firing was similar in rats ($n = 3$) of a different strain (Sprague-Dawley; not illustrated). In controls with i.v. injection of saline, no significant change in substantia nigra pars reticulata firing was seen (Fig. 2A, B).

Following i.p. administration of 30 mg/kg gabapentin, firing rate of substantia nigra pars reticulata neurons decreased in the 3 rats used at this dosage (Fig. 3A), but

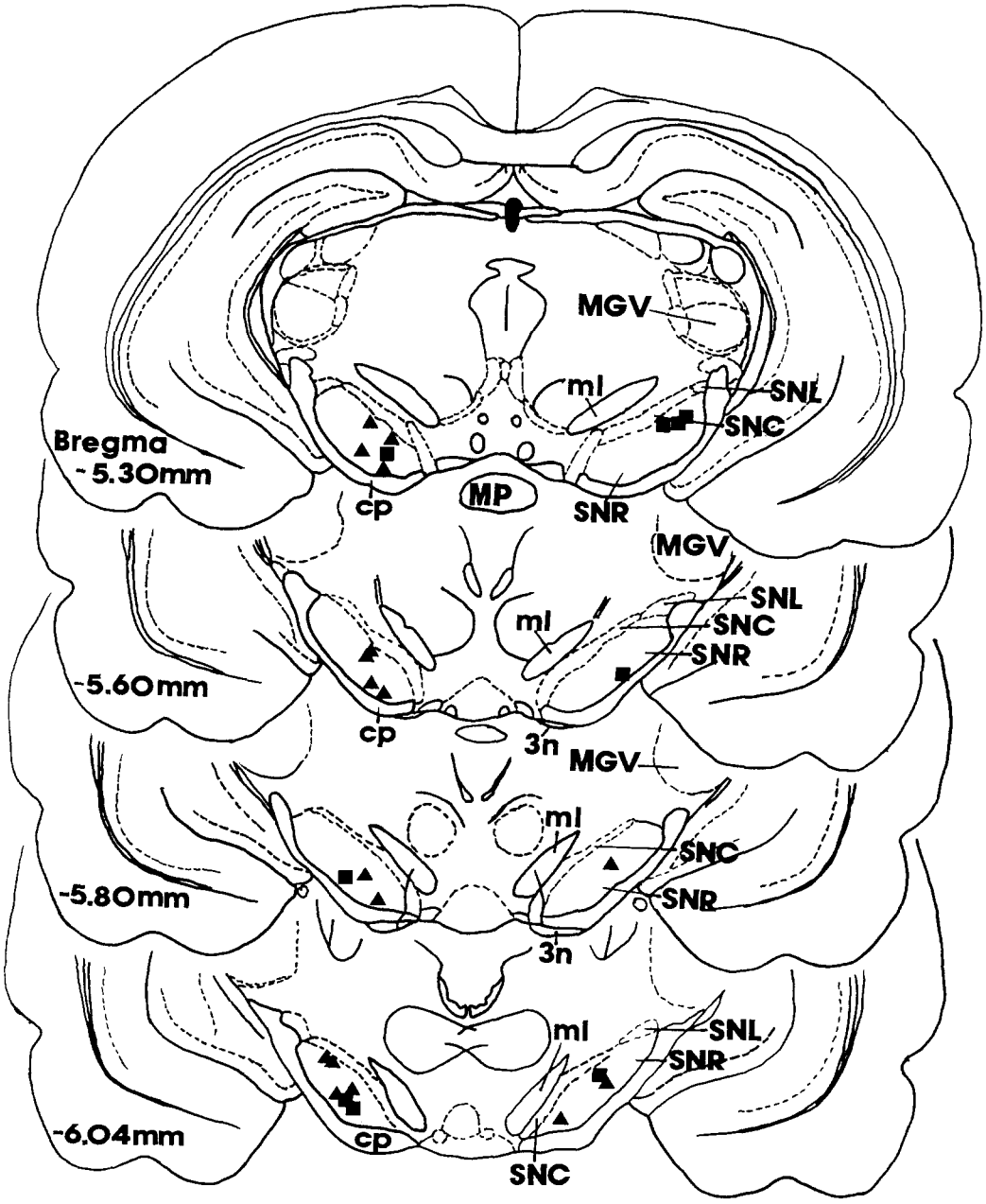

Fig. 1. Location of horseradish peroxidase spots in the substantia nigra pars reticulata as plotted on drawings of coronal brain sections according to the stereotaxic atlas of Paxinos and Watson (1986). Locations from vehicle control are indicated by squares and locations from gabapentin experiments by triangles. Each symbol represents one animal. Abbreviations: 3n, occulomotor nerve or its root; cp, cerebral peduncle; ml, medial lemniscus; MGV, medial geniculate nucleus, ventral part; MP, medial mammillary nucleus, posterior part; SNC, substantia nigra pars compacta; SNL, substantia nigra pars lateralis; SNR, substantia nigra pars reticulata.

onset of gabapentin's action on substantia nigra pars reticulata firing was retarded compared to i.v. injection. Whereas with i.v. injection, a significant decrease of neuronal activity was seen as rapid as 4 min after injection (Fig. 2A), a lag time of at least 15 min passed before neuronal firing decreased after i.p. injection (Fig. 3A).

In order to examine the dose-dependency of gabapentin's effect on single unit firing of substantia nigra pars reticulata neurons, a lower dose of 15 mg/kg was administered i.v. in 4 rats (Fig. 3B). No consistent alteration in substantia nigra pars reticulata activity was seen at this dosage. Increase of dose to 20 mg/kg (tested in 1 rat) resulted in a marked reduction of substantia nigra pars reticulata activity by 60% compared to predrug baseline (not illustrated).

A comparison of baseline firing rate of individual substantia nigra pars reticulata neurons with the individual inhibitory effect of gabapentin did not indicate any relationship between these two variables at any dosage, i.e., neurons with a high firing rate did not differ in their response to drug administration from neurons with lower firing rates. Furthermore, there was no relationship between the location of a neuron within substantia nigra pars reticulata (see Fig. 1) and its response to drug application.

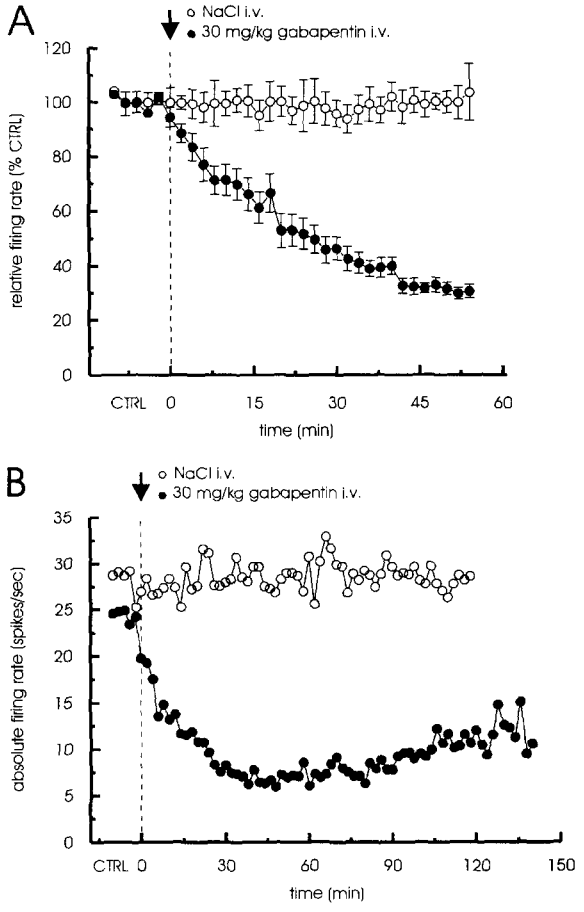

Fig. 2. Effect of i.v. administration of vehicle (saline) and gabapentin (30 mg/kg) on spontaneous firing of substantia nigra pars reticulata neurons. Data in the upper graph (A) are means ± S.E. of 6 Wistar rats per group and are shown relative to the mean baseline (control; ctrl) firing rates of each group. Each symbol refers to a recording period of 2 min. Average recordings are shown up to the longest recording period which was available for all rats per group. The time scale below the recordings shows the recording period in min before and after drug administration. Time of drug or vehicle injection is indicated by the arrow and the vertical hyphenated line. Statistical comparison of vehicle control and drug data indicated that following 4 min after drug administration and at all subsequent time points substantia nigra pars reticulata activity was significantly lower (P at least < 0.05) than in vehicle control. The lower graph (B) illustrates absolute firing rates of substantia nigra pars reticulata neurons in two Wistar rats, one injected with vehicle and the other with gabapentin, 30 mg/kg i.v. These animals were chosen from the group values in (A) because of the long duration of recording in these rats.

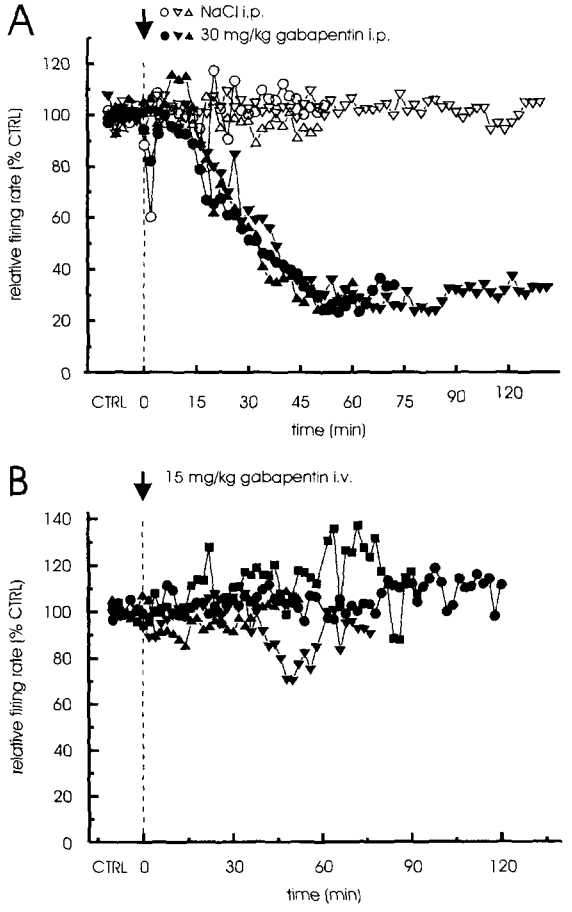

Fig. 3. Effect of vehicle and different doses of gabapentin on spontaneous firing of substantia nigra pars reticulata neurons. Data in the upper graph (A) are from experiments with i.p. administration of vehicle (3 Wistar rats) or 30 mg/kg gabapentin (3 Wistar rats) and are shown relative to the mean baseline (control; ctrl) firing rates of each group. Each symbol refers to a recording period of 2 min. The time scale below the recordings shows the recording period in min before and after drug administration. Time of drug or vehicle injection is indicated by arrow and the vertical hyphenated line. The lower graph (B) illustrates relative firing rates of substantia nigra pars reticulata neurons in 4 Wistar rats after i.v. administration of 15 mg/kg gabapentin.

4. Discussion

The present study demonstrates that gabapentin, administered systemically at an anticonvulsant dose in rats, significantly inhibits the spontaneous firing of substantia nigra pars reticulata neurons in a manner similar to that previously described for the anti-epileptics valproate, phenobarbital, and benzodiazepines (Waszczak et al., 1986; Löscher et al., 1995; Rohlfs et al., 1996). It is important to note that inhibition of substantia nigra pars reticulata firing is not a common effect of anti-epileptics but is restricted to those drugs which are known to facilitate GABAergic transmission, either by an effect on the $GABA_A$ receptor complex (barbiturates, benzodiazepines) or by increasing GABA turnover in substantia nigra pars reticulata (valproate) (Waszczak et al., 1986; Löscher, 1993). It is likely that GABAergic transmission in the substantia nigra pars reticulata acts as a seizure-gating mechanism to control seizure propagation in various distinct epileptogenic circuits including those for generating limbic seizures and generalized convulsive as well as non-convulsive seizures (Gale, 1988, 1992; Löscher and Ebert, 1996). Thus, pharmacological potentiation of GABA function in the substantia nigra pars reticulata represents an effective strategy to obtain protection against a broad spectrum of seizures (Gale, 1992; Löscher and Ebert, 1996). Gabapentin prevents seizures from a variety of different electrical and chemical stimuli and is also active in several genetic models of epilepsy, as well as in patients with partial seizures (Löscher and Schmidt, 1994; Chadwick, 1995; Taylor, 1995). It is thus tempting to speculate that the proven efficacy of gabapentin as a novel anti-epileptic drug is a function of its marked effect on neuronal activity in the nigra.

The substantia nigra pars reticulata contains almost exclusively GABAergic neurons which are under inhibitory influence of GABAergic terminals arising from GABAergic neurons in the striatum (Gale, 1988). When excitation predominates in the striatum, the striatonigral GABA pathway is activated, thereby inhibiting GABAergic neurons in the substantia nigra pars reticulata. This in turn leads to disinhibition of target neurons of the GABAergic substantia nigra pars reticulata output pathways, which is associated with an inhibition of seizure propagation through these circuits, i.e., a seizure-gating effect (Gale, 1988). This effect might be promoted by an activation of the external globus pallidus which also sends inhibitory GABAergic projections to substantia nigra pars reticulata (Fallon and Loughlin, 1995). Microinjection and lesion studies suggest that this gating influence of the substantia nigra pars reticulata is exerted predominantly via disinhibition of neurons in the deep layers of the superior colliculus (Gale, 1988). Interestingly, GABA synthesis and/or $GABA_A$ receptor function in substantia nigra pars reticulata are impaired in some models of focal and generalized epilepsy, indicating that a deficient gating influence of the substantia nigra pars reticulata is involved in the pathogenesis of different seizure types (Löscher and Schwark, 1985; Olsen et al., 1986). Consistent with this view, Bonhaus et al. (1986, 1991) reported that substantia nigra pars reticulata neurons enter an intense burst-firing pattern during focal seizures in kindled but not in non-kindled rats, suggesting that kindling-induced epileptogenesis is associated with a change in the intrinsic properties of the substantia nigra pars reticulata. Increase of GABA turnover in substantia nigra pars reticulata by drugs such as valproate (Löscher, 1989) and gabapentin (Löscher et al., 1991) and the resulting decrease in substantia nigra pars reticulata firing could thus constitute an effective means of restoring or potentiating the seizure-gating function of the substantia nigra pars reticulata. Interestingly, the dose (30 mg/kg) of gabapentin found effective in the present experiments to decrease firing of substantia nigra pars reticulata neurons was the lowest effective anticonvulsant dose in kindled rats (Taylor, 1995).

After our initial observation that gabapentin increases GABA turnover (Löscher et al., 1991), several subsequent studies reported that gabapentin interacts with the GABA system. The drug was shown to increase the activity of the GABA synthesizing enzyme glutamic acid decarboxylase; (Taylor et al., 1992) which could explain the increased GABA turnover in response to gabapentin (Löscher et al., 1991). Furthermore, gabapentin was shown to increase GABA release in rat brain slices and isolated optic nerve tissue, possibly by reversal of GABA transport (Götz et al., 1993; Kocsis and Honmou, 1994; Honmou et al., 1995a,b; Fichter et al., 1996). Recently, Petroff et al. (1996) reported in vivo measurements of GABA in human brain by magnetic resonance spectroscopy, indicating that gabapentin increases brain GABA in patients with complex partial seizures. However, as shown by previous studies with GABA elevating drugs, it is certainly not a global increase in brain GABA which is important for seizure protection, but increase of GABA turnover and release in certain discrete and highly specific brain regions, such as substantia nigra pars reticulata, in which enhancing GABA transmission exerts an anticonvulsant action (Gale, 1988).

In addition to increasing GABA turnover and release, gabapentin has been demonstrated to exert several other effects which could be involved in its anticonvulsant action, including interactions with some cytosolic enzymes of brain tissue, binding to the system L neutral amino acid transporter, and reduction of sustained repetitive firing of sodium-dependent action potentials of central neurons (cf., Taylor, 1995). However, it is presently unclear whether these actions are necessary for the anticonvulsant effects of gabapentin.

Interestingly, the onset of gabapentin's effects on single unit firing of substantia nigra pars reticulata neurons in the present experiments in rats closely matched its onset of anticonvulsant action in this species (Taylor, 1995, and

unpublished experiments with i.v. and i.p. administration of gabapentin in the maximal electroshock seizure test). Despite the rapid onset of anticonvulsant activity, peak anticonvulsant effects after i.v. or i.p. injection are reached not before about 2 h and lag behind peak drug concentrations in brain tissue and microdialysate (Vollmer et al., 1986; Welty et al., 1993, and unpublished data). Pharmacokinetic experiments have shown that gabapentin crosses the blood–brain barrier very rapidly and reaches maximal brain levels within 30–60 min (Vollmer et al., 1986; Welty et al., 1993). Because of this rapid penetration into the brain, it has been suggested that gabapentin is transported by an active transport process (Welty et al., 1993).

With respect to drug potency, gabapentin was recently reported to block maximal electroshock seizures at 15 mg/kg i.v. in about 80% of male Sprague–Dawley rats (Welty et al., 1993), while no consistent effect on substantia nigra pars reticulata neuronal firing was observed at this dose in the present experiments. In this respect it should be noted that maximal electroshock seizure experiments with gabapentin in female Wistar rats as used in the present neurophysiological experiments indicated that doses of at least 20–30 mg/kg are needed to exert significant anticonvulsant effects (W. Löscher and D. Hönack, unpublished experiments), which matches the dose range found effective to decrease substantia nigra pars reticulata activity in this strain and gender.

In conclusion, the present study is a further, obviously important step toward establishing a cellular mechanism of action for gabapentin. Gabapentin's prevention of seizures from GABA antagonists or GABA synthesis inhibitors in rodents (Bartoszyk et al., 1986), findings of increased GABA synthesis in vivo (Löscher et al., 1991) together with findings of increased GABA release from brain slices (Götz et al., 1993; Honmou et al., 1995a,b) and the present finding of in vivo inhibition of neuronal firing in the substantia nigra pars reticulata, where protection against a broad spectrum of seizures can be obtained by increasing GABA function (Gale, 1988), indicate that enhancement of GABAergic mechanisms is a reasonable hypothesis for the anticonvulsant action of gabapentin. In this respect, gabapentin resembles the widely used anti-epileptic valproate, which has also been shown to increase GABA release, glutamic acid decarboxylase activity and nigral GABA turnover, and to inhibit substantia nigra pars reticulata firing, though the precise cellular mechanisms involved in these effects of valproate might differ, at least in part, from those of gabapentin (Löscher, 1993). However, it is important to note that, although gabapentin and valproate seem to exert similar effects on the GABA system, they differ in a number of important aspects. For instance, in contrast to valproate, gabapentin is ineffective against nonconvulsive absence-like seizures in a genetic rat model and against photosensitive, myoclonic seizures in baboons (Taylor, 1995). These differences strongly suggest that valproate has additional mechanisms of anticonvulsant action different from gabapentin (cf., Löscher, 1993).

Acknowledgements

We thank Dr. Ulrich Ebert and Dr. Anke Rohlfs (Battelle Europe, Geneva) for helpful advice during the electrophysiological measurements and Dr. Charles P. Taylor (Parke-Davis Research, Ann Arbor, MI, USA) for providing gabapentin.

References

Adams, J.C., 1981, Heavy metal intensification of DAB-based HRP reaction product, J. Histochem. Cytochem. 29, 775.

Bartoszyk, G.D., N. Meyerson, W. Reimann, G. Satzinger and A. von Hodenberg, 1986, Gabapentin, in: New Anticonvulsant Drugs, eds. B.S. Meldrum and R.J. Porter (John Libbey, London) p. 147.

Bonhaus, D.W., R.D. Russell and J.O. McNamara, 1991, Activation of substantia nigra pars reticulata neurons: Role in the initiation and behavioral expression of kindled seizures, Brain Res. 545, 41.

Bonhaus, D.W., J.R. Walters and J.O. McNamara, 1986, Activation of substantia nigra neurons: Role in the propagation of seizures in kindled rats, J. Neurosci. 6, 3024.

Bunney, B.S., J.R. Walters, R.H. Roth and G.K. Aghajanian, 1973, Dopaminergic neurons: Effect of antipsychotic drugs and amphetamine on single cell activity, J. Pharmacol. Exp. Ther. 185, 560.

Chadwick, D. 1995, Gabapentin. Clinical use, in: Anti-epileptic Drugs, Vol. 4, eds. R.H. Levy, R.H. Mattson and B.S. Meldrum (Raven Press, New York, NY) p. 851.

Fallon, J.H. and S.E. Loughlin, 1995, Substantia nigra, in: The Rat Nervous System, Vol. 2, ed. G. Paxinos (Academic Press, New York, NY) p. 215.

Fichter, N., C.P. Taylor and T.J. Feuerstein, 1996, Nipecotate-induced GABA release from slices of the rat caudato-putamen: Effects of gabapentin, Naunyn-Schmiedeberg's Arch. Pharmacol., in press.

Gale, K. 1988, Progression and generalization of seizure discharge: Anatomical and neurochemical substrates, Epilepsia 29 (Suppl. 2), S15.

Gale, K. 1992, GABA and epilepsy: Basic concepts from preclinical research, Epilepsia 33 (Suppl. 5), S3.

Götz, E., T.J. Feuerstein and D.K. Meyer, 1993, Effects of gabapentin on release of gamma-aminobutyric acid from slices of rat neostriatum, Arzneim.-Forsch. (Drug Res.) 43, 636.

Guyenet, P.G. and G.K. Aghajanian, 1978, Antidromic identification of dopaminergic and other output neurons of the rat substantia nigra, Brain Res. 150, 69.

Honmou, O., J.D. Kocsis and G.B. Richerson, 1995a, Gabapentin potentiates the conductance increase induced by nipecotic acid in CA1 pyramidal neurons in vitro, Epilepsy Res. 20, 193.

Honmou, O., A.A. Oyelese and J.D. Kocsis, 1995b, The anticonvulsant gabapentin enhances promoted release of GABA in hippocampus: A field potential analysis, Brain Res. 692, 273.

Kocsis, J.D. and O. Honmou, 1994, Gabapentin increases GABA-induced depolarization in rat neonatal optic nerve, Neurosci. Lett. 169, 181.

Löscher, W. 1989, Valproate enhances GABA turnover in the substantia nigra, Brain Res. 501, 198.

Löscher, W. 1993, Effects of the anti-epileptic drug valproate on metabolism and function of inhibitory and excitatory amino acids in the brain, Neurochem. Res. 18, 485.

Löscher, W. and U. Ebert, 1996, Basic mechanisms of seizure propagation: Targets for rational drug design and rational polypharmacy, Epilepsy Res. (Suppl.), in press.

Löscher, W. and D. Schmidt, 1994, Strategies in anti-epileptic drug development: Is rational drug design superior to random screening and structural variation? Epilepsy Res. 17, 95.

Löscher, W. and W.S. Schwark, 1985, Evidence for impaired GABAergic activity in the substantia nigra of amygdaloid kindled rats, Brain Res. 339, 146.

Löscher, W., D. Hönack and C.P. Taylor, 1991, Gabapentin increases aminooxyacetic acid-induced GABA accumulation in several regions of rat brain, Neurosci. Lett. 128, 150.

Löscher, W., A. Rohlfs and C. Rundfeldt, 1995, Reduction in firing rate of substantia nigra pars reticulata neurons by valproate: Influence of different types of anesthesia in rats, Brain Res. 702, 133.

Olsen, R.W., J.K. Wamsley, R.J. Lee and P. Lomax, 1986, Benzodiazepine/barbiturate/GABA receptor–chloride ionophore complex in a genetic model for generalized epilepsy, in: Basic Mechanisms of the Epilepsies. Molecular and Cellular Approaches, eds. A.V. Delgado-Escueta, A.A.J. Ward, D.M. Woodbury and R.J. Porter (Raven Press, New York, NY) p. 365.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates (Academic Press, Sydney).

Petroff, O.A.C., D.L. Rothman, K.L. Behar, D. Lamoureux and R.H. Mattson, 1996, The effect of gabapentin on brain gamma-aminobutyric acid in patients with epilepsy, Ann. Neurol. 39, 95.

Rohlfs, A., C. Rundfeldt, R. Koch and W. Löscher, 1996, A comparison of the effects of valproate and its major active metabolite E-2-en-valproate on single unit activity of substantia nigra pars reticulata neurons in rats, J. Pharmacol. Exp. Ther. 277, 1305.

Simons, D.J. and P.W.A. Land, 1987, A reliable technique for marking the location of extracellular recording sites using glass micropipettes, Neurosci. Lett. 81, 100.

Taylor, C.P., M.G. Vartanian, R. Andruszkiewiewicz and R.B. Silverman, 1992, 3-Alkyl GABA and 3-alkylglutamic acid analogues: Two new classes of anticonvulsant agents, Epilepsy Res. 11, 103.

Taylor, C.P. 1995, Gabapentin. Mechanisms of action, in: Anti-Epileptic Drugs, Vol. 4, eds. R.H. Levy, R.H. Mattson and B.S. Meldrum (Raven Press, New York, NY) p. 829.

Vollmer, K.O., A. von Hodenberg and E.U. Kolle, 1986, Pharmacokinetics and metabolism of gabapentin in rat, dog and man, Arzneim.-Forsch. (Drug Res.) 36, 830.

Waszczak, B.L., E.K. Lee and J.R. Walters, 1986, Effects of anticonvulsant drugs on substantia nigra pars reticulata neurons, J. Pharmacol. Exp. Ther. 239, 606.

Welty, D.F., G.P. Schielke, M.G. Vartanian and C.P. Taylor, 1993, Gabapentin anticonvulsant action in rats: Disequilibrium with peak drug concentrations in plasma and brain microdialysate, Epilepsy Res. 16, 175.