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Development of Tolerance to the Anticonvulsant Effect of GABAmimetic Drugs in Genetically Epilepsy-Prone Gerbils

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LOSCHER, W *Development of tolerance to the anticonvulsant effect of GABAmimetic drugs in genetically epilepsy-prone gerbils* PHARMACOL BIOCHEM BEHAV 24(4) 1007–1013, 1986 —Three drugs which increase GABA-mediated inhibitory neurotransmission in the brain, namely the GABA degradation inhibitors aminooxyacetic acid (AOAA) and γ-acetylenic GABA (GAG), and the GABA receptor agonist THIP (gaboxadol), were administered to epilepsy-prone gerbils via subcutaneously implanted osmotic minipumps for 2 weeks The antiepileptic drugs valproic acid (VPA) and diazepam were also included in the experiments After one day of constant rate application, all GABAmimetics markedly suppressed seizure activity induced in the gerbils by air blast stimulation, but anticonvulsant efficacy of the drugs was lost after 8 and 14 days of treatment With VPA, only moderate anticonvulsant effects were found because only sub-therapeutic drug levels (about 40 μg/ml plasma) were reached via minipump administration The experiments with diazepam could only be evaluated in part because of instability of the drug in aqueous solution Determination of brain GABA metabolism in the gerbils indicated that reduction of GABA synthesis may be responsible, at least in part, for development of tolerance to the anticonvulsant effects of AOAA and GAG

GABAmimetic drugs Valproic acid Diazepam Epilepsy-prone gerbils Air blast stimulation Osmotic minipumps Anticonvulsant effects Tolerance GABA metabolism

DESPITE innumerable studies on the primary mechanisms underlying epileptic phenomena, the neurochemical and electrophysiological processes that cause and maintain epilepsy in humans are still unknown. The Mongolian gerbil (*Meriones unguiculatus*) offers a unique model for the investigation of the role of neurotransmitters in the genesis and maintenance of epileptic activity because it represents a mammalian nervous system with a genetic disorder of brain excitability (cf. [20]). Epilepsy-prone gerbils display paroxysmal electroencephalographic and motor activity in response to various external stimuli, including change in environment [28], handling [12], and exposure of the animals to a blast of compressed air [7]. Studies in epileptic gerbils on various drugs which selectively modify the activity of excitatory and inhibitory neurotransmitters in the brain have shown that seizures in gerbils are strikingly sensitive to GABAmimetic drugs, i.e., compounds that selectively increase GABA-mediated inhibitory neurotransmission [21,27]. Accordingly, two recent neurochemical reports indicate that alterations in GABAergic neurotransmission may be involved in the seizure-prone state in this species [31,35]. In view of the fact that several GABAmimetic drugs are currently undergoing clinical trials in a number of neurological disease states, including epilepsy [2, 13, 33, 36], we felt it important to study the effects of prolonged treatment with such drugs on seizure behaviour in epilepsy-prone gerbils. The following drugs were included in this study: aminooxyacetic acid (AOAA) and γ-acetylenic GABA (GAG), which increase brain GABA concentrations by irreversible inhibition of GABA degradation [16], THIP (4, 5, 6, 7-tetrahydroisoxazolo [5,4-c] pyridin-3-ol; gaboxadol), a selective and potent GABA receptor agonist [13], and valproic acid (VPA), a clinically established antiepileptic drug which increases central GABA concentrations in different species, including humans, by a yet unsettled effect on GABA metabolism [22]. Drugs were administered in gerbils via subcutaneously implanted osmotic minipumps which delivered respective drug solutions continuously at a constant rate for two weeks, and effects on seizure behaviour and GABA metabolism were studied. Similar experiments carried out with the antiepileptic drug diazepam could only be evaluated in part because of drug instability during the administration period.

METHOD

Animals

Randomly bred gerbils of both sexes were provided by Bundesgesundheitsamt (Federal Bureau of Health, Berlin) or purchased from Hoechst AG (Frankfurt, F.R.G.) The

TABLE 1

EFFECT OF PROLONGED TREATMENT WITH THIP, AOAA, GAG AND VPA ON GABA METABOLISM IN EPILEPTIC GERBILS

Drug	Daily dose (mg/kg)	No of gerbils	Body weight before treatment	Body weight after 14 days of treatment	Brain GABA metabolism after 14 days of treatment: GABA (μmol/g)	GAD (μmol/g/h)	GABA-T (μmol/g/h)
None	—	10	70 ± 6 3	72 ± 5 2	2 2 ± 0 3	20 ± 5 6	44 ± 3 4
THIP	30 ± 1 7	8	74 ± 6 1	77 ± 4 9	1 9 ± 0 7	16 ± 0 8	41 ± 8 7
AOAA	8 7 ± 0 9	8	69 ± 7 0	68 ± 5 4	4 3 ± 1 3‡	15 ± 2 3*	28 ± 10‡
GAG	7 9 ± 0 6	8§	75 ± 6 7	77 ± 6 1	2 9 ± 0 8†	14 ± 1 7†	37 ± 3 6‡
VPA	480 ± 35	8	65 ± 3 9	67 ± 2 3	2 3 ± 0 4	16 ± 3 3	49 ± 7 2

Drugs were administered via osmotic minipumps for 14 days, in control animals saline-filled pumps were implanted subcutaneously For each animal, the daily dose of drug was calculated by determining the difference between the initial loading of the pump and the amount of drug solution remaining in the pump after the 14 day treatment period Data for daily dose, body weight and GABA metabolism given in the table are means ± S D of the number of gerbils indicated

*$p<0$ 05, †$p<0$ 01, ‡$p<0$ 001, §1 animal died after 13 days of treatment (not included in data for daily dose, body weight and GABA metabolism)

animals were housed singly in plastic cages at constant temperature (24–26°C) and controlled humidity (50%) with a 12-hr light cycle beginning at 7:00 a.m. and were supplied with food (Altromin 1324 standard diet; Altromin, Lage, F R G) and water ad lib The gerbils were stimulated once weekly in the morning by exposure to a blast of compressed air (average pressure 5 bars) aimed at the back of the animals for 15 sec in a separate empty plastic cage. Details of this technique and classification of seizures stimulated thereby have been described elsewhere [8,24] Fifty animals which had shown reproducible grade 5 seizures (generalized tonic-clonic seizures with loss of righting reflexes) in response to air blast for at least 4 consecutive weeks were used for the present experiments. Gerbils showing seizures in response to change in environment or handling (<10% of the animals of our colony) were not included

Implantation of Osmotic Minipumps

Gerbils were anesthetized by methohexital (Brevimytal, 35 mg/kg IP) and osmotic minipumps capable of continuously delivering drug solutions for 2 weeks (series 2ML2, Alza Co , Palo Alto, CA) were implanted subcutaneously into the back of the animals In each gerbil, one pump was placed into a pocket which was opened after a small incision had been made in the pelvic region. The pump was inserted with the flow moderator pointing away from the incision. The minipump consisted of an impermeable flexible drug reservoir surrounded by a thin sleeve of osmotic agent which was encapsuled by a semipermeable membrane The drug reservoir was filled with aqueous solutions of VPA (250 mg of the sodium salt per ml), AOAA (5 mg of the hydrochloride per ml, adjusted to pH 6 by dilute NaOH), GAG (5 mg/ml) and THIP (17.5 mg of the hydrate per ml), respectively Net weight of the solution loaded into each pump was determined by the difference in weight between the empty and filled pump. Fill volumes varied between 1.9–2 4 g and care was taken that these volumes were always over 90% of the reservoir volumes specified by the manufacturer for the individual pumps. The concentrations of drug solutions were chosen on the basis of previous experiments in gerbils on the anticonvulsant and pharmacokinetic characteristics of the respective drugs after acute administration [7,27] to give daily doses of about 500 mg/kg VPA, 10 mg/kg AOAA or GAG and 35 mg/kg THIP, taking into account the mean pumping rates given by the manufacturer of the pumps. All animals received bolus injections of the different drugs immediately after implantation of the pumps (100 mg/kg VPA, 2 mg/kg AOAA, GAG or THIP) in a volume of 5 ml/kg IP Eight animals were used for each drug Experiments were also carried out with diazepam (2.5 ml per ml, brought into solution by dilute HCl; bolus injection with 1 mg/kg IP), but this drug turned out to be not stable in the pumps during the administration period (see below) Ten control gerbils were implanted with saline-filled minipumps and received a bolus injection of 5 ml/kg saline After implantation of the pumps, the incisions were closed by sutures. The pumps were activated by water imbibed by the osmotic agent through the semipermeable membrane The imbibed water caused swelling of the osmotic agent which forced the drug solution out of the reservoir through the flow moderator into the subcutaneous area in a continuous and controlled manner The pumping rates were determined by the difference between fill volume and volume remaining in the pump reservoirs after 14 days, and these figures were used for calculation of the daily doses of drugs per kg of body weight (see Table 1). Pumping rates thus determined varied between 4 3 and 5 8 μl of drug solution per hour, which corresponded very well to the pumping rates given by the manufacturer, indicating that all 50 minipumps had worked with sufficient accuracy

Seizure Testing

Previous experiments with the gerbils of our colony have shown that, once developed, grade 5 seizures can be evoked in most animals very reproducibly for months or even years provided 1 week elapses between each stimulation. If the interval between 2 stimulations is less than 4 days, seizure severity is decreased or even no seizures can be induced, indicating a very long lasting refractory period after each seizure (unpublished experiments). Thus, for the present experiments, intervals between stimulations of 6–7 days were

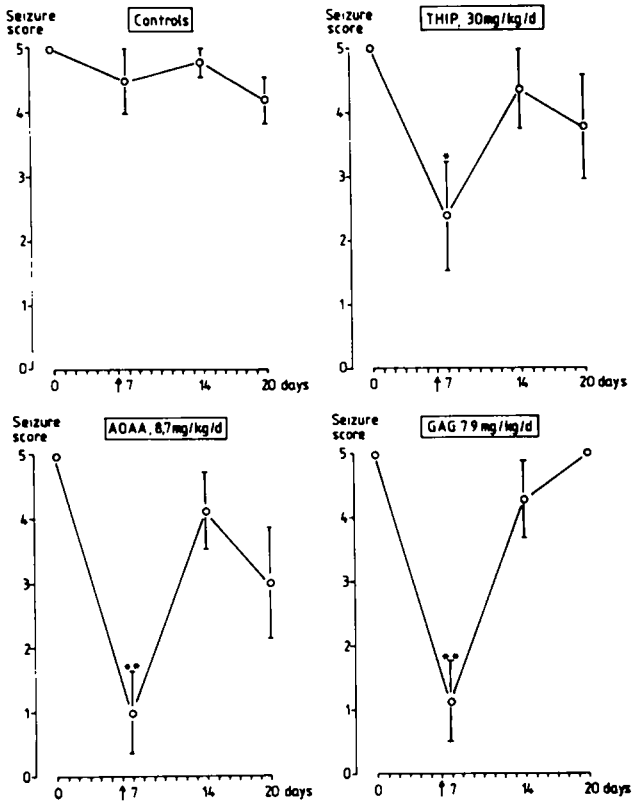

FIG 1 Effect of chronic treatment with THIP, AOAA and GAG on seizure severity in epilepsy-prone gerbils with grade 5 (generalized tonic-clonic) seizures in response to air blast stimulation Drugs were administered in groups of 8 gerbils via osmotic minipumps for 14 days (for detailed daily doses see Table 1). A group of 10 gerbils with saline-filled minipumps served as controls. For each group, the day on which control experiments were performed prior to implantation of minipumps is indicated on the abscissa by "0;" the arrows indicate the time of pump implantation Data are means±S E , absence of S E indicates that all animals had identical seizure scores Significance of differences between treated groups and the control group is marked by asterisks (*$p<0$ 01, **$p<0$ 001)

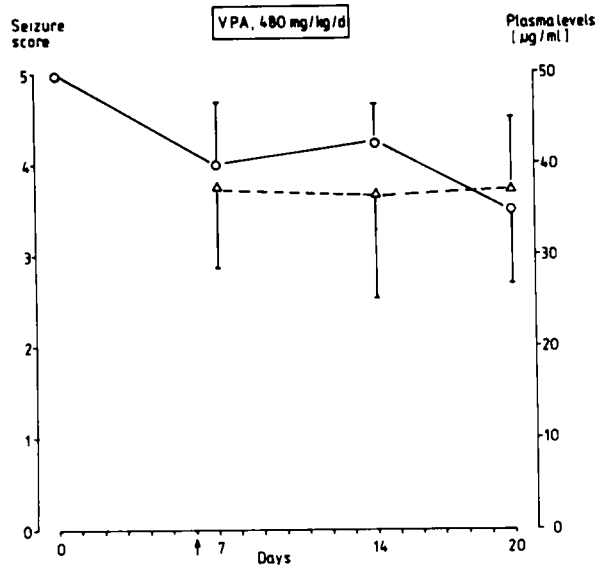

FIG 2 Effect of chronic treatment with VPA on seizure severity in epilepsy-prone gerbils with grade 5 (generalized tonic-clonic) seizures in response to air blast stimulation. VPA was administered to 8 gerbils via osmotic minipumps for 14 days (for detailed daily dose see Table 1). The day on which control experiments were performed prior to implantation of minipumps is indicated on the abscissa by "0;" the arrow indicates the time of pump implantation. Data for seizure severity (O—O) are means±S E of the 8 animals, absence of S.E. indicates that animals had identical readings. Mean seizure scores during VPA treatment did not significantly differ from those determined in control animals (See Fig 1) Plasma levels of VPA (△—△) are means±S E of 4–8 animals

used. Osmotic minipumps were implanted in seizure-prone gerbils 6 days after the last control stimulation by air blast. One day after the implantation, i.e., 7 days after the control stimulation, gerbils were stimulated in the morning by compressed air and seizure response was graded according to the scale described by Loskota *et al.* [28]. This procedure was repeated 8 and 14 days following implantation of the minipumps. During all stimulations, the experimenter was not aware of which animals were treated and which were controls. Significance of differences in mean seizure scores between control animals and treated ones was calculated for each experimental day by Student's *t*-test.

Drug Determinations

In the VPA and diazepam treated groups, blood was sampled by orbital puncture for drug analysis in plasma. For this purpose, 4 animals of each group were punctured 1 day after implantation of the minipumps and 4 other animals after 7 days of drug administration. 0.5 ml blood was withdrawn from each gerbil Following 14 days of treatment, all animals were killed by decapitation and blood was collected from the VPA and diazepam treated groups for drug analysis. VPA was measured in plasma by gas chromatography with flame ionisation detection [14], and diazepam and its metabolites desmethyldiazepam and oxazepam were analyzed by gas chromatography with electron capture detection [16]. The same analytical methods were also used to determine stability of the aqueous VPA and diazepam solutions loaded into the osmotic minipumps by comparing drug concentrations in the initial loading and in the residual volume remaining in the pump after the 14 days of the experiment. Stability of AOAA, GAG and THIP in aqueous solutions at body temperature was examined by drug determination after derivative formation using a newly developed gas chromatographic method. Briefly, small aliquots of the respective aqueous drug solutions were placed into 2-ml glass vials and evaporated to dryness by a steam of nitrogen. To the drug residue were added 25 mg of anhydrous potassium carbonate and 0.5 ml of a 0.4% solution of pentafluorobenzyl bromide (Fluka AG, Buchs, F.R.G.). The vials were sealed, heated at 60°C for 1 hr, and the content then evaporated to dryness by nitrogen. To the dried residue was added 0 5 ml of distilled water, which was shaken for 5 min. Then, 0.5 ml of hexane was added and the mixture was shaken for 10 min. 5 µl-aliquots of the hexane layer were directly injected into a Varian 3700 gas chromatograph equipped with a flame ionisation detector. The column was a silanized 6-ft glass tubing with 2-mm inner diameter packed with 3% SP-2250 on Supelcoport

100–120 mesh Nitrogen was used as carrier gas at a flow rate of 40 ml/min The analysis was carried out at an initial column temperature of 110°C for 1 min, which was raised by 40°/min to 270° and maintained at this temperature for 5 min The temperatures of injector and detector were at 300° and 350°C, respectively. Under these conditions, AOAA, GAG and THIP appeared as sharp, symmetrical peaks with retention times of 3 8, 4 8 and 7 5 min, respectively. Known amounts of AOAA were used as internal standard for quantification of GAG and THIP, whereas GAG was used as internal standard for determination of AOAA

Neurochemical Determinations

After the last air blast stimulation, i e., 14 days following implantation of the pumps, all gerbils were killed by decapitation and the brains were rapidly removed, divided by a sagittal section in the midline into two equal portions and weighed. One brain half was immediately homogenized in 80% ethanol for determination of GABA, whereas the other half was homogenized in distilled water (containing 0.05% Triton X-100, 1 mM 2-mercaptoethanol and 0 1 mM pyridoxal phosphate) for determination of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD, EC 4 1 1 15) and the GABA degrading enzyme GABA aminotransferase (GABA-T, EC 2 6 1.19). GABA was determined by the enzymatic "GABAase" method and GAD and GABA-T by fluorimetric techniques as previously described for gerbil brain [27] Arithmetical means and S D are given for the neurochemical determinations Significance of differences between treated groups and controls was calculated by Student's *t*-test

Drugs

VPA, used as the sodium salt, was a gift from Desitin-Werk Carl Klinke (Hamburg, F R.G.) Diazepam was obtained from Hoffmann-La Roche (Grenzach/Whylen, F R G) THIP H_2O was kindly provided by H Lundbeck & Co A/S (Copenhagen, Denmark) and GAG by Centre de Recherche Merrell International (Strasbourg, France). AOAA hemihydrochloride was purchased from Sigma (Munich, F R G) Drugs were dissolved in saline except diazepam, which was dissolved in distilled water by means of dilute HCl

RESULTS

Drug Stability

In order to be sure that alterations in drug efficacy during constant-rate application of aqueous drug solutions via osmotic minipumps was not secondary due to instability of drug solutions at body temperature, drug concentrations were determined in the solutions by gas chromatography. The results showed that VPA, AOAA, GAG and THIP were stable in aqueous solutions at body temperature for 14 days, whereas diazepam concentrations decreased by about 70% Because of this instability, the experiments on diazepam could only be evaluated in part (see below).

General Behaviour of the Gerbils During Prolonged Drug Administration

Following implantation of osmotic minipumps, animals recovered rapidly and no marked changes in general behaviour were noted one day after the surgery During the 14-day treatment periods with the different drugs, all animals except one (see below) behaved normally, showed their usual exploratory activity, and no alterations in body weight were noted (see Table 1) Only one gerbil of the group treated with GAG progressively lost weight, showed a marked decrease in body temperature, ptosis, ataxia and died after 13 days of treatment

Seizure Behaviour of the Gerbils During Prolonged Drug Administration

Results are shown in Fig 1 and 2 In controls, the mean seizure score was not altered significantly by implantation of the minipumps during the course of the experiment, although a moderate decrease in seizure severity was noted (Fig 1) In the gerbils treated with THIP, AOAA and GAG, seizure severity was decreased significantly after 1 day of constant-rate application (Fig 1) In fact, 5 animals were completely protected from seizures by AOAA and GAG and 3 animals by THIP, and most of the remaining gerbils exhibited only grade 1–2 seizures However, after 8 and 14 days of treatment, this marked anticonvulsant effect of the GABAmimetics had disappeared, thus indicating development of tolerance

During treatment with VPA (Fig 2), only moderate, non-significant decreases in seizure severity were obate, however, in contrast to the GABAmimetic drugs, the moderate anticonvulsant effect of VPA seemed to increase with time, 2 of the 8 animals becoming seizure-free after 14 days of treatment. Average plasma concentrations of VPA remained very stable during the treatment period, ranging between 37 and 40 μg/ml

After one day of treatment with diazepam, mean seizure score was decreased from 5 to 3 1 (2 animals became seizure-free) which, however, was not significantly different from controls (not illustrated) Average plasma concentrations of diazepam and its metabolites desmethyldiazepam and oxazepam were 41, 5 and 19 ng/ml, respectively Drug concentrations in plasma and the effect on seizure severity were reduced at the subsequent experimental days, which was certainly due to the instability of diazepam solution in the minipumps (see above), although tolerance to diazepam may also have been involved in the loss of efficacy

Brain GABA Metabolism in Gerbils During Prolonged Drug Administration

Brain GABA concentrations as well as GAD and GABA-T activities in controls and drug-treated gerbils after the 14-day treatment period are shown in Table 1 In gerbils on treatment with AOAA and, less marked, GAG, GABA concentrations were increased significantly as a result of significant inhibition of GABA-T However, in both groups the activity of GAD was also decreased significantly Following THIP and VPA, no significant changes in GABA metabolism were determined The diazepam treated group was also not different from controls in this respect (not illustrated)

DISCUSSION

The present study demonstrates that tolerance develops to the anticonvulsant effect of different GABAmimetic drugs in seizure-prone gerbils, thus confirming previous studies with GABAmimetics in mice [19] In fact, during treatment of mice with the GABA-T inhibitors GAG, γ-vinyl GABA

(GVG) and ethanolamine-O-sulphate (EOS) via the drinking water for 12 days, there was a significant increase in the threshold for maximal (tonic extension) electroconvulsions after 4 days, but this anticonvulsant effect was reduced or lost after 8 and 12 days in spite of further accumulation of brain GABA [19]. Similarly, Sykes *et al* [38] found no effect on electroshock convulsions in rats treated with EOS for 7, 14 and 24 days despite large increases in brain GABA concentrations. In agreement with these reports, the present data in gerbils show that the GABA-T inhibitors AOAA and GAG had lost their anticonvulsant effect after 8 and 14 days of continuous administration despite GABA concentrations in the brain were significantly increased. Whereas different studies thus suggest that, at least in rats, mice and gerbils, the anticonvulsant action of GABA-T inhibitors is reduced or lost during prolonged treatment, the present experiments indicate for the first time that tolerance also develops to the anticonvulsant effect of GABA receptor agonists, such as THIP.

The mechanisms underlying the development of tolerance to the anticonvulsant effect of different categories of GABAmimetic drugs seem to differ. For both GABA-T inhibitors and THIP, there is no evidence for metabolic tolerance, i.e , enhanced metabolic inactivation of the drugs that leads to progressively lower drug levels during chronic treatment [6, 13, 19]. For comparison, administration of the antiepileptic drug phenobarbital via osmotic minipumps in gerbils was found to result in marked induction of microsomal liver enzymes so that it was not possible to maintain active drug levels during treatment [8] Studies with GABA-T inhibitors in mice have shown that the loss in anticonvulsant activity of these drugs during continued treatment is paralleled by significant reductions in brain GAD activity, i.e., the enzyme that catalyses the formation of GABA [19] GAD is thought to exert direct control over the GABA pool involved in neurotransmission and reduced activity of this enzyme might actually lower the amounts of GABA available for release into the synaptic cleft, even though overall brain GABA content is elevated [1] Indeed, in the present experiments with AOAA and GAG in gerbils, significant reductions in GAD activity were found after the 14-day treatment period. GAG is known to exert a direct inhibitory effect on GAD activity after administration in rodents, including gerbils [11, 17, 27], whereas AOAA does not seem to inhibit the enzyme directly *in vivo*[17,23] In fact, reduction of GAD activity during prolonged administration of GABA-T inhibitors which do not directly inhibit GAD *in vivo* (AOAA, EOS, GVG) is thought to reflect a feedback repression of *de novo* synthesis of GAD by the accumulation of brain GABA [6, 19, 37] Alternatively, reduction of GAD activity in the presence of elevated GABA levels could also relate to the recent finding that GABA is capable of inactivating GAD by converting it to the apoenzyme [34] Besides possible feedback effects of highly elevated GABA levels on GAD, there is considerable evidence for the presence of presynaptically located GABA autoreceptors which, when activated, inhibit GABA release from GABAergic nerve terminals [3,30]. Some degree of decrease of GAD activity and/or GABA release may thus be an inevitable consequence of elevating brain GABA concentrations over a critical value, which clearly limits the anticonvulsant effectiveness of GABA-T inhibitors

A further explanation for progressive reduction in anticonvulsant activity in spite of high GABA levels in the brain would be an alteration in the sensitivity of postsynaptic GABA receptors. Studies on GABA-T inhibitors in this respect are controversial. Chronic elevation of brain GABA by AOAA or GAG in rats was found to lead to subsensitivity of GABA receptors in the corpus striatum, whereas receptor sensitivity remained unchanged in frontal cortex, hippocampus and cerebellum [4,5]. It is not clear why only the striatal GABA receptors were desensitized since GABA levels are increased in all regions ob the brain following treatment with AOAA and GAG [10,26], but alterations in dopaminergic activity in the striatum may have been involved In contrast to the findings with AOAA and GAG, prolonged treatment with EOS increased GABA binding in rat cortex, due to an increase in the number of GABA binding sites [38]. One possible explanation for increased GABA binding after EOS is that this compound, in contrast to other GABA-T inhibitors, exerts weak direct agonistic action at GABA receptors [15,32] which in case of high receptor occupancy may lead to a compensatory up-regulation of these receptors [38]. Chronic elevation of brain GABA by GVG had no significant effect on GABA binding in the cerebral cortex of rats, but other brain regions were not examined [9].

In contrast to AOAA and GAG, no alterations in GABA metabolism were observed following prolonged administration of THIP in gerbils. The most likely explanation for development of tolerance to the anticonvulsant effect of this potent GABA agonist would be a down-regulation of GABA receptors. In fact, prolonged administration of THIP in mice at daily doses of 5 mg/kg for 14 days has recently been shown to result in a decrease in the binding sites for GABA in the cortex and hippocampus [37]. Alternatively, a reduction of GABA release may be involved in the loss of anticonvulsant efficacy, since THIP had been shown to inhibit GABA release from rat cortical synaptosomes, most probably via an effect on presynaptic autoreceptors for GABA [3]. Irrespective of the mechanisms responsible for the marked tolerance observed in gerbils, it appears from the present data that THIP is not a valuable new drug for treatment of epilepsy. In fact, in a preliminary clinical study in which THIP was administered to 9 epileptic patients for 6 months, no significant antiepileptic effect could be determined [33].

Whereas the present experiments indicated that constant-rate application of drugs via osmotic minipumps in epilepsy-prone gerbils is a valuable means for determination of the long-term efficacy of anticonvulsant drugs, the drawbacks of this method also became apparent. With valproic acid, only sub-therapeutic plasma levels were reached and maintained ("therapeutic" plasma concentration range in humans is 50–100 μg/ml; [22]) although daily doses of about 500 mg/kg were administered. Similar findings were reported for administration of VPA via osmotic minipumps in rats [25] which demonstrates that these devices are less suited for the prolonged administration of drugs, such as VPA, with very short half-lives (half-life of VPA in gerbils is 0 7 hr, [7]). and low anticonvulsant potency, because preparation of sufficiently high concentrated drug solutions to reach effective levels in the animal is limited by the solubility of the respective drug The present lack of significant effects of VPA on seizure severity and GABA metabolism in gerbils can be explained in this way. For comparison, in acute experiments VPA was shown to block seizures in gerbils with an ED 50 of 70 mg/kg IP [27], which gives VPA plasma concentrations of about 200 μg/ml at the time of testing (unpublished experiments)

Another important limitation of administration of drugs via minipumps was demonstrated by the experiments with

diazepam, which proved to be not stable in aqueous solution at body temperature Thus, determination of drug stability should precede or accompany experiments in which drugs are administered for longer time periods via osmotic minipumps before definite conclusions about the long-term efficacy of drugs administered in this way can be drawn

In conclusion, refuting a recent report in gerbils [29], the present experiments show that seizure-prone gerbils are a valuable natural model of epilepsy for long-term investigation of anticonvulsant drugs provided the pharmacokinetics of the drugs and their stability is taken into consideration The present data on continued treatment of epilepsy-prone gerbils with GABAmimetic drugs confirm previous experiments in mice and rats in that tolerance develops to the anticonvulsant effect of drugs which induce large increases in brain GABA levels by irreversible inhibition of GABA-T Furthermore, the data provide evidence that the anticonvulsant effect of the potent GABA agonist THIP is also lost during prolonged treatment, indicating that development of tolerance is not restricted to one class of GABAmimetic drugs Tolerance to the anticonvulsant effect of GABAmimetic drugs is most likely caused by the development of compensatory mechanisms within the GABA system, such as reduction of GABA synthesis and release, and desensitization of postsynaptic GABA receptors Induction of such negative feedback effects may be less marked with drugs, such as VPA and the GABA receptor agonist progabide, which are much weaker in their GABAmimetic action than drugs such as THIP, GAG or AOAA In fact, with VPA and progabide no tolerance to the anticonvulsant effect seems to develop in epileptic patients [2,22] The present findings may be of relevance for the possible therapeutic potential of different GABA-T inhibitors and GABA agonists, especially in view of the fact that at present the effectiveness of such drugs in patients with epilepsy is a matter of controversy [2, 13, 33, 36]

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