Table 3. Effect of trimethoprim (TMP) infusion on simultaneous renal tubular excretion of tetraethylammonium (TEA) and *p*-aminohippuric acid (PAH)

Infusion	Tetraethylammonium ATEF	Tetraethylammonium % Recovery/min	*p*-Aminohippuric acid ATEF	*p*-Aminohippuric acid % Recovery/min
Substrates alone (control)	0.536 ± 0.147	92.0 ± 5.9	0.59 ± 0.04	106.8 ± 17.7
Substrates + TMP	0.115 ± 0.044*	63.2 ± 3.4*	0.55 ± 0.07	92.7 ± 7.4

Each value is the mean ± SD from three experiments. Recovery is defined as the total amount excreted/min from both kidneys ÷ total amount infused/min. Infusion rates (μmol/min): TEA, 0.01; PAH, 0.01; and TMP, 0.10. ATEF = apparent tubular excretion fraction.
* Significantly different ($P < 0.05$) from control.

value of TEA was reduced by 78.6%, whereas that of PAH was not changed significantly (−6.3%). Additionally, TMP selectively reduced the urinary recovery of TEA without significantly affecting that of the PAH. These data are consistent with the conclusion that TMP is secreted by the organic cation transport system.

In summary, this study has produced the following three principal conclusions: (1) TMP was actively transported *in vivo* in the excretory direction by the renal tubules, (2) the renal tubular cells did not metabolize TMP as it crossed from peritubular blood to tubular lumen, and (3) the active transport occurred via the organic cation system. Thus, a number of cationic drugs and endogenous chemicals are potential competitors for the active tubular excretion of TMP.

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Postictal refractoriness associated with reduction of glutamic acid decarboxylase in discrete brain regions in epilepsy-prone gerbils

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It has been known for a long time that experimentally-induced seizures results in a refractory state during which the animal will not respond to previously effective seizure-precipitating stimuli [1–5]. It is possible that this postictal refractoriness represents an adaptation of the brain to diminish or prevent the spread of further seizure activity [4]. The mechanisms involved in producing this postictal state are not known, but numerous studies suggest that the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), may be involved. Thus, transient increases in GABA concentrations have been observed in different brain regions of rats following a seizure induced by electroshock or bicuculline [6]. The increase observed in the hippocampus was closely related temporally to the increase in seizure threshold following the initial convulsion. Marked increases of GABA in hippocampus and other brain regions were also observed after seizure activity induced by kainic acid and L-allylglycine in rats [7]. An indication of increased GABAergic function was also reported by Ross and Craig [8], who found an enhanced GABA receptor binding in the cortex after an electroshock seizure in rat, and by Shin *et al.* [9], who found increases in GABA binding in rat hippocampus following an amygdala-kindled seizure. Furthermore, several groups observed increases in the binding of benzodiazepines at the GABA/benzodiazepine receptor complex following a seizure induced by electroshock or pentylenetetrazol in rats [10], by amygdala-stimulation in kindled rats [11, 12] and by handling in epilepsy-prone gerbils [13, 14]. The increase in benzodiazepine receptor binding in the postictal period was shown to be accompanied by increased inhibition using electrophysiological measurements [15]. However, although attractive, the relationship between postictal refractoriness and increased GABAergic inhibition is not straightforward, because biochemical indices for altered GABAergic function are not found after all types of generalized seizures and some workers [6] could not reproduce the postictal increase in benzodiazepine binding reported by others (see above). Furthermore, more recently Green and co-workers [16–18] found decreases in GABA synthesis and release following an electroshock- or flurothyl-induced seizure, which seems to contradict the GABA hypothesis of postictal refractoriness. In the present study, we investigated the time course of postictal depression in seizure-prone Mongolian gerbils, a genetically predisposed species in which seizures can be initiated by different sensory stimuli

(cf. ref 19). At the time of maximum postictal refractoriness, we determined the activity of glutamic decarboxylase (GAD; EC 4.1.1.15), i.e. the rate-limiting enzyme in the synthesis of GABA, as an index for presynaptic activity of the GABA system.

Materials and methods

From the age of 7–8 weeks, randomly bred gerbils of both sexes were tested once a week for seizure-sensitivity and intensity of the seizures. The testing procedure involved placing the gerbil into an empty plastic cage and exposing the animal to a blast of compressed air (average pressure 5 bars) aimed at the back of the gerbil for 15 sec. Details of this technique and classification of seizures stimulated thereby have been described elsewhere [19]. In brief, seizure severity was rated as follows: 0 = no seizure; 1 = twisting of vibrissae and pinnae, animal still moving normally; 2 = motor arrest with more pronounced twitching of vibrissae, ears and eyelids; 3 = motor arrest with generalized myoclinic jerks; 4 = generalized clonic–tonic seizures without loss of righting reflexes; 5 = generalized clonic–tonic seizures with loss of righting reflexes. For the present study, we used only animals which displayed consistent grade 5 seizures for several weeks prior to the experiments. The time course of postictal refractoriness after a grade 5 seizure was determined in two ways: (a) In groups of 10–15 gerbils, air blast stimulation was repeated at different times after a grade 5 seizure and seizure severity was rated as described above. Each group was only used for one time point after the initial seizure. (b) In groups of 15 gerbils, the threshold for maximal (tonic extension) electroconvulsions was determined at different times after a grade 5 seizure. The electroconvulsive threshold was determined via eye electrodes with the "up and down" method [20] as described previously for mice [21] by means of a Lafayette A-615 B shocker with the following stimulation data: a.c. of 50 cycles/sec for 0.2 sec with the serial resistance of the apparatus set to 10 kΩ. The electroconvulsive threshold was expressed as the voltage necessary to induce a tonic hind limb extension in 50% of the animals (EV_{50}). Each group of animals was only used for one threshold determination after the air blast-induced grade 5 seizure. Animals, in which the threshold was determined one week after a grade 5 seizure, served as controls. Repeated determination of this control threshold in different groups of gerbils showed no significant variation in this measure from group to group (data not illustrated).

For the biochemical determinations, two groups of six animals each were used. One group was sacrificed by decapitation one week after the last air blast-induced grade 5 seizure, the other group 15 min after the seizure. In order to avoid interassay variation error, samples from the two groups were treated concurrently in all phases of the neurochemical analysis. Brains were rapidly removed and dissected on a cold plate at −18° into 11 brain regions as previously described for rat brain [22]. The individual brain regions were then rapidly homogenized in 1 ml of ice-cold distilled water containing 1 mM 2-mercaptoethanol, 0.1 mM pyridoxal phosphate, and 0.5% Triton X-100. The activity of GAD was measured according to the fluorimetric method of Lowe *et al.* [23] as described previously [24]. In a final volume of 1.0 ml, the incubation medium contained 5 mM L-glutamic acid, 0.24 mM pyridoxal phosphate, 100 mM phosphate buffer (pH 6.4), and 0.5 ml of the tissue homogenate. Protein was measured by the method of Lowry *et al.* [25] as modified by Markwell and colleagues [26].

Significance of differences between seizure severity scores was calculated by the Wilcoxon signed rank test for paired replicates. For statistical evaluation of seizure threshold and GAD data, the unpaired Student's *t*-test was used.

Results and discussion

It has been reported previously that daily sensory stimulation of seizure-susceptible gerbils results in a marked diminution of the seizure susceptibility, whereas stimulation of the same animals at weekly intervals yields a reproducible seizure incidence [27]. Subsequent studies in this species showed that repeated seizures at intervals of 4 days or less result in a refractory state during which the animal will not respond to previously effective seizure precipitating stimuli [28, 29]. The data shown in Fig. 1 demonstrate that seizure susceptibility to air blast stimulation drops rapidly after a grade 5 seizure. Mean seizure severity was significantly reduced from grade 5 to about 3 from 15 min to 2 hr after the initial seizure. The extent of this reduction in seizure susceptibility varied considerably from animal to animal. The maximum effect was observed after 15 min at which three gerbils exhibited no seizure activity, four animals showed grade 4 seizures, while three animals exhibited grade 5 seizures in response to the air blast stimulus. Mean seizure severity gradually returned towards control values after the first 2 hr following the initial seizure, and after 24 hr all animals exhibited grade 5 seizures again. Thus, the progressive reduction of seizure susceptibility reported with repeated daily stimulation in gerbils [27] may be due to an accumulation of the postictal events which were observed here.

Two recent studies have shown that 15 min following a seizure in gerbils, there is a significant increase in the threshold for seizures induced by pentylenetetrazol [30, 31] or electroshock [31]. The data given in Fig. 2 demonstrate that the time course of this postictal increase in seizure threshold differs somewhat from that of the reduction in susceptibility to sensory stimulation shown in Fig. 1. The maximum increase in threshold was determined 1 hr after a single grade 5 seizure, and significant threshold elevation was maintained for up to 24 hr after the seizure. Thus, the duration of the postictal rise in seizure threshold in gerbils is considerably longer than that of the seizure threshold rise observed after single electroshock- or bicuculline-induced seizures and amygdala-kindled seizures in rats [32, 33].

The results of the GAD determinations in gerbils one week or 15 min after a grade 5 seizure are shown in Fig. 3. Compared to the GAD data in the absence of postictal refractoriness, i.e. one week after the seizure, GAD activities at 15 min after the seizure tended to be lower in all brain regions except cerebellum and medulla. The differences between both groups were significant in corpus striatum, thalamus and hypothalamus. These data thus seem to substantiate the findings of Green *et al.* [16, 18] with electroshock- and flurothyl-induced seizures in rats that GABA synthesis is decreased in the postictal period. As already mentioned, following an electroshock seizure increases in GABA concentrations associated with decreases of GABA synthesis and release have been reported by Green's group [6, 16–18], which would seem to indicate a functional reduction of GABAergic transmission. However, a more likely explanation for reduction in GABA turnover and release is that these effects occur in response to the increase in GABA/benzodiazepine receptor sensitivity which has been found after a single electroshock seizure [8, 10]. Similar increases in postsynaptic GABA/benzodiazepine receptor sensitivity have also been reported to occur in the refractory period of other experimentally-induced seizures [9–13] and it was shown that these alterations are associated with an enhanced postsynaptic GABAergic function [15].

Interpretation of the meaning of the present findings in gerbils for the postictal state in this species is complicated by the fact that significant decreases in GAD activity were only determined in brain regions (striatum, thalamus, hypothalamus) which are predominantly involved in brain functions unrelated to epilepsy, whereas in regions, such as substantia nigra, tectum and hippocampus, which seem to

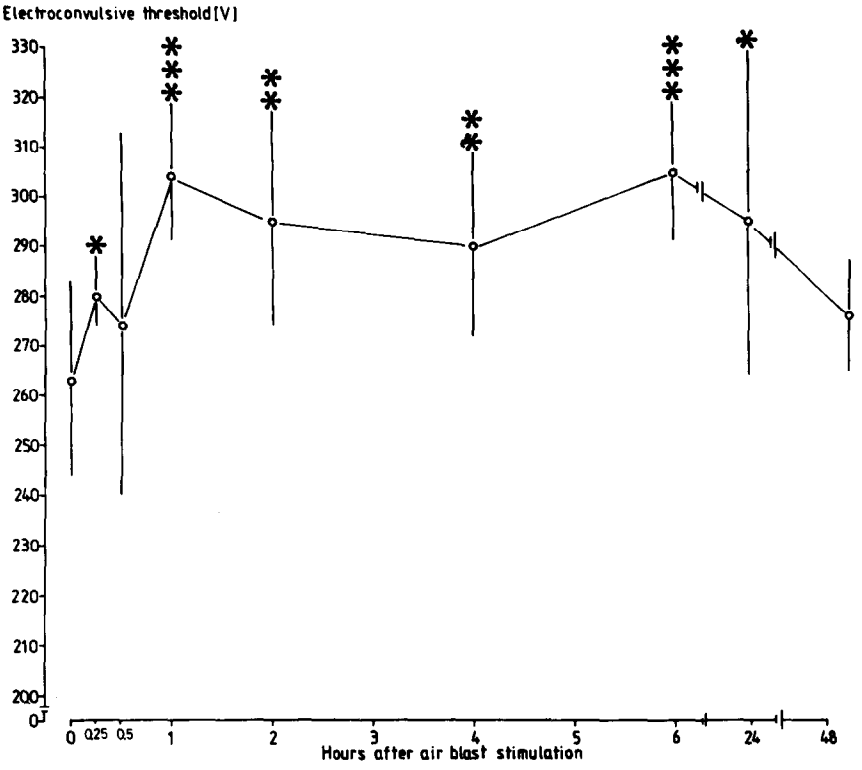

Fig. 1. Postictal refractoriness in seizure-susceptible gerbils with grade 5 (generalized clonic–tonic) seizures in response to air blast stimulation. Each symbol gives the mean seizure score (± SE) of a group of 10–15 gerbils which were stimulated by air blast at the time indicated after an air blast-induced grade 5 seizure. Each group was used only for one time point after the initial seizure so that a total of 85 gerbils was used for these experiments. Absence of SE indicates that all animals had identical seizure severity scores. Significance of difference to the initial seizure severity is indicated by asterisks (*$P < 0.05$; **$P < 0.01$).

be directly associated with seizure genesis and/or propagation [34, 35], no significant alterations in GAD were observed. However, it should be considered that recent studies in rats on GAD activities in whole tissue and nerve terminals (synaptosomes) of different brain regions, including substantia nigra, tectum and hippocampus, have shown that significant decreases in GAD activity at the nerve terminal level (where GABA is predominantly synthesized) are, at least in part, concealed if GAD is only determined in whole tissue of the respective region [36]. This may also explain the findings of Green *et al.* [16–18], who determined marked postictal decreases in GABA synthesis in brain regions (hippocampus, striatum and cortex) of rats after an electroshock seizure, but they were unable to detect any change in whole tissue GAD activity of these regions. Thus, GABA turnover, release and/or postsynaptic receptor function may be more reliable indices for GABAergic function than whole tissue GAD activity. With respect to GABA receptor function in gerbils, a significant increase in binding of benzodiazepines at the GABA/benzodiazepine/chloride ionophore receptor complex has been determined 5 min after a seizure in substantia nigra, cortex and interpeduncular nucleus when data were compared to those of seizure-sensitive gerbils prior to the seizure [14]. Similarly, Asano and Mizutani [13] reported increases in benzodiazepine binding in gerbils 10 min after a handling-induced grade 4 or 5 seizure in cerebral cortex, hippocampus, brain stem and striatum, but not cerebellum [13]. The most remarkable increase was seen in the striatum, in which we found significant reduction of GAD activity at 15 min after the seizure. Thus, the GAD reduction reported here most probably represents a compensatory reaction to a postsynaptic increase in GABAergic neurotransmission. In fact, facilitation of GABAergic transmission by benzodiazepines is known to be associated with a reduction of GABA synthesis [37, 38]. However, the postictal refractoriness in gerbils is not related simply to an increase in postsynaptic GABA function, since in the experiments of Asano and Mizutani [13] benzodiazepine binding in gerbils returned to the control level already 20 min after the seizure, whereas we found a reduced seizure susceptibility for at least 2 hr. It should be considered that this discrepancy could be due to the different seizure induction techniques used in both studies. Indeed, air blast is a stronger seizure precipitating stimulus in gerbils than is handling [19].

Irrespective of the data on GABAergic transmission, previous studies of Lee and Lomax [30] have suggested that postictal refractoriness in gerbils may be mediated by endogenous opioids; however, as recently reported [31], we could not reproduce their experimental results. Various other transmitter systems, including monoamines, acetylcholine, adenosine, and prostaglandins, have been implicated in the postictal rise of seizure threshold, but no clear evidence for a pivotal role of any of these transmitters has been obtained yet [4, 5]. In any event, the most likely explanation for postictal refractoriness is a transient activation of endogenous anticonvulsant processes which may be responsible for spontaneous seizure arrest as well as suppression of further seizures. Numerous results including the present suggest that GABAergic transmission is involved in this phenomenon.

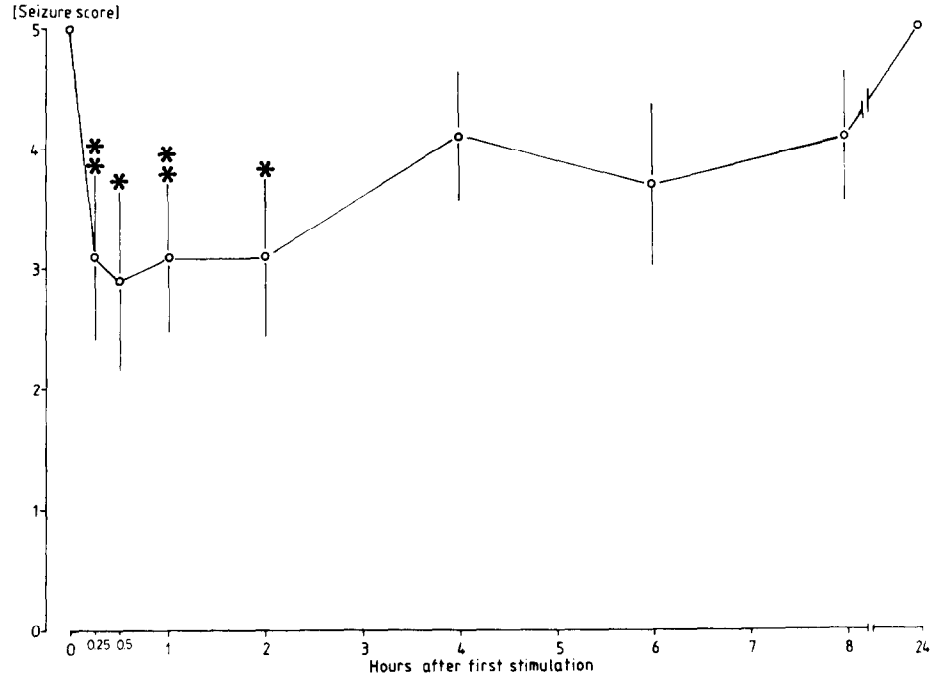

Fig. 2. Postictal rise in seizure threshold in seizure-susceptible gerbils with grade 5 seizures in response to air blast stimulation. Each symbol represents the threshold for tonic electroconvulsions (EV_{50} with confidence limits for 95% probability) in a group of 15 gerbils at different times after an air blast-induced grade 5 seizure. Each group was only used for one threshold determination. At the time indicated as "0", the control threshold determined 1 week after a previous grade 5 seizure, i.e. in the absence of postictal refractoriness, is shown. Significance of differences to this control threshold is indicated by asterisks (*$P < 0.02$; **$P < 0.01$; ***$P < 0.001$).

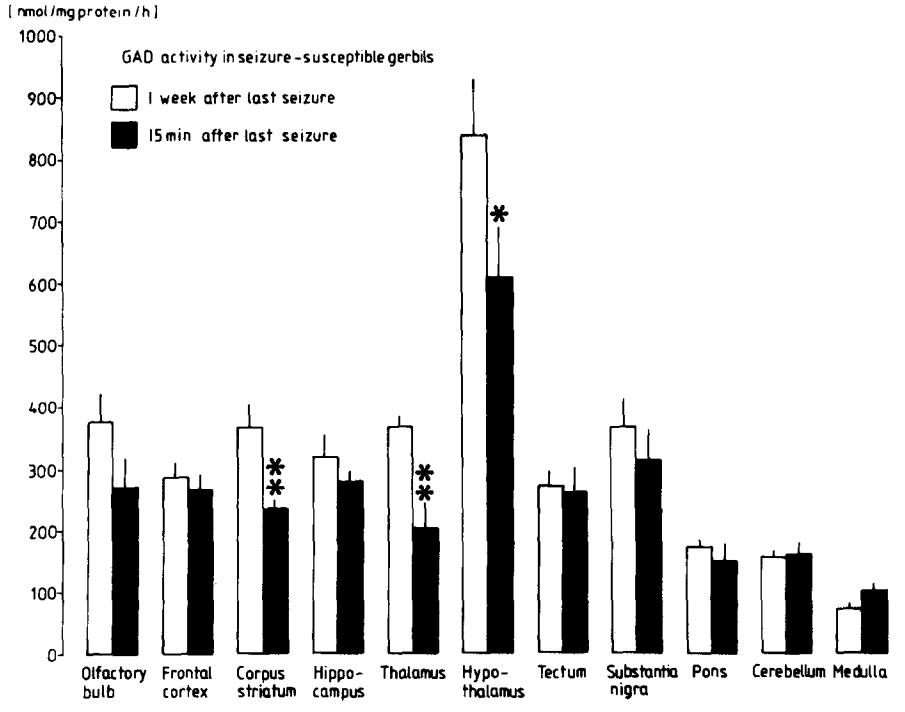

Fig. 3. Activity of the GABA-synthesizing enzyme GAD in different brain regions of seizure-susceptible gerbils 15 min or 1 week after an air blast-induced grade 5 seizure. Data are means ± SE of 6 gerbils per group. Significance of differences between both groups is indicated by asterisks (*$P < 0.05$; **$P < 0.01$).

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