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Carbamazepine pharmacokinetics–pharmacodynamics in genetically epilepsy-prone rats

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Abstract

Carbamazepine produces dose-related anticonvulsant effects in epilepsy models including the genetically epilepsy-prone rat (GEPR) model and the rat maximal electroshock model. Dose–response relationships are quantitatively different among the models. Against electroshock seizures in Sprague–Dawley rats the ED_{50} dose is 7.5 mg/kg whereas the ED_{50} against audiogenic seizures in severe seizure GEPRs (GEPR-9s) is 3 mg/kg. In contrast, the ED_{50} in moderate seizure GEPRs (GEPR-3s) is 25 mg/kg. The present study was designed to ascribe dose–response differences among the three rat strains to pharmacokinetic or pharmacodynamic factors. After systemic carbamazepine, pharmacokinetic studies revealed differences in area under the concentration-vs.-time curve. In other experiments, carbamazepine-induced serotonin release from hippocampus was used as a pharmacodynamic marker. In a concentration-controlled design using intracerebral microdialysis, hippocampal carbamazepine infusions produced similar concentration–response relations for the three rat strains. These data support the hypothesis that dose–response differences among the three rat strains are primarily pharmacokinetic in nature.

Keywords: Carbamazepine; Anticonvulsant; Epilepsy; Genetically epilepsy-prone rat; Microdialysis; Hippocampus; Focal administration; Seizure; Pharmacokinetics; Pharmacodynamics

1. Introduction

Antiepileptic drugs produce a variety of cellular actions that appear to be related to their capacity to suppress seizures (see Rogawski and Porter, 1990; White, 1997 for reviews). Several antiepileptic drugs including carbamazepine (Yan et al., 1992; Kaneko et al., 1993; Dailey et al., 1997a,b), valproate (Whitton and Fowler, 1991), antiepilepsirine (Yan et al., 1992), loreclezole (Dailey et al., 1994), zonisamide (Okada et al., 1992; Kaneko et al., 1993), and lamotrigine (Southam et al., 1998) increase extracellular levels of the inhibitory neurotransmitter serotonin. For carbamazepine, the increases in extracellular serotonin are dose-related in Sprague–Dawley rats (Dailey et al., 1997b) and genetically epilepsy-prone rats (GEPRs) (Dailey et al., 1997a). In both rat strains, the carbamazepine doses required to decrease convulsion intensity also increase serotonin release.

The GEPR model of epilepsy consists of two rat strains selectively and independently bred (see Reigel et al., 1986 for a description of GEPR strains development). The response to sound stimulation was the behavioral endpoint used to select breeders that led to the GEPR strains. In one strain, audiogenic seizures are moderate (generalized clonus with loss of righting reflex), while in another strain, they are severe (generalized tonic/clonic convulsions very similar to those seen with maximal electroshock). According to the Audiogenic Response Score system developed by Jobe et al., 1973 for evaluation of seizure severity, moderate seizure GEPRs exhibit class 3 seizures, whereas severe seizure GEPRs exhibit class 9 seizures. Thus, moderate seizure GEPRs are frequently described as GEPR-3s and severe seizure GEPRs as GEPR-9s.

Seizure predisposition in GEPRs manifests as susceptibility to seizures induced by stimuli that fail to cause seizures in neurologically normal animals. Besides their susceptibility to audiogenic seizures, GEPRs exhibit a low incidence of spontaneous seizures and adult GEPR-9s experience hyperthermic seizures (Dailey et al., 1989).

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Generally, hyperthermic seizures occur in non-epileptic animals only during the early stages of postnatal development. In addition, GEPRs demonstrate increased sensitivity to most chemoconvulsants and exhibit both increased sensitivity and an extended range of convulsive responses to electrical stimuli (Browning et al., 1990).

All clinically useful antiepileptic drugs tested and several experimental compounds decrease audiogenic seizure severity in both types of GEPRs (Dailey and Jobe, 1985; Dailey et al., 1989, 1992; Yan et al., 1992; Dailey et al., 1994, 1995). However, not all drugs are equally effective anticonvulsants in GEPR-3s and GEPR-9s. For example, GEPR-3s require significantly larger doses of carbamazepine or phenytoin to experience an anticonvulsant effect than do GEPR-9s. Each strain is protected by approximately equal doses of clonazepam, antiepilepsirine and phenobarbital. Finally, GEPR-9s require significantly larger doses than do GEPR-3s to experience an anticonvulsant effect with valproate, loreclezole or D-20443 (Dailey and Jobe, 1985; Dailey et al., 1989, 1994, 1995).

GEPR-9s experience an anticonvulsant effect in response to lower doses of carbamazepine than do GEPR-3 or Sprague–Dawley rats (Dailey and Jobe, 1985). GEPR-9s also exhibit larger increases in brain extracellular serotonin in response to lower doses of carbamazepine than do GEPR-3 or Sprague–Dawley rats (Dailey et al., 1997a,b). The present study had the objective to assess carbamazepine pharmacokinetic differences between GEPR-3s and GEPR-9s in an attempt to explain the aforementioned pharmacodynamic differences.

2. Material and methods

2.1. Animals

The present studies were conducted in three groups of rats. The two test groups compared 200–380 g GEPR-9s and GEPR-3s. The GEPRs came from breeding stock maintained at the University of Illinois College of Medicine at Peoria. The control group contained 250–350 g, female Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN, USA).

2.2. Pharmacokinetic studies

Animals achieved anesthesia after i.p. injections of ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg). Under sterile conditions, the jugular vein was isolated. One end of a polyethylene catheter was introduced into the jugular vein. The other end was tunneled subcutaneously to exit through an incision in the middle back. Next, the animals were secured in a Kopf stereotaxic instrument. A microdialysis guide cannula tip (BAS, West Lafayette, IN, USA) was placed at the following coordinates relative to bregma: anterior–posterior = −5.2 mm, lateral = 4.2 mm, depth = 7 mm from top of skull (Paxinos and Watson, 1986). The guide cannula was affixed to the animal's skull with dental acrylic anchored by machine screws. Animals were removed from the stereotaxic instrument and clothed in a jacket to protect the subcutaneous catheters.

Animals received two microdialysis probes after overnight recovery. The jugular probe was a loop microdialysis probe composed of cellulose acetate hollow fibers (i.d. 215 ± 15 μm, molecular weight cutoff = 6000 Da, Spectrum Medical Industries, Los Angeles, CA, USA). The brain probe (4 mm membrane, BAS) was inserted into the brain guide cannula with the tip 2 mm below the end of the guide cannula. The jugular probe had an active dialysis membrane 3.2 cm in length. When the loop type probe was folded onto itself, the active dialysis area was 1.6 cm long. The jugular probe was inserted in the jugular catheter so the probe tip extended 1–2 cm beyond the catheter toward the heart. Separate Teflon™ tubes (o.d. = 0.65 mm, i.d. = 0.12 mm, BAS) connected the microdialysis probes to infusion pumps and fraction collectors. Each animal was attached to wide elastic bands and suspended within a Plexiglas™ chamber. The elastic bands permitted unimpeded limb movement but prevented twisting and turning that might disarticulate the connecting tubes.

A solution of 2-methyl-carbamazepine (5 pmol/μl, Aldrich, Milwaukee, WI, USA) in artificial cerebrospinal fluid perfused the brain microdialysis probe. Artificial cerebrospinal fluid had the following solute concentrations: Na^{2+} 150 mM, K^{+} 3.0 mM, Ca^{2+} 1.2 mM, Mg^{2+} 0.8 mM, and Cl^{-} 155 mM. For a perfused brain area, the dialysis probe delivered drug to and removed solute from a cylindrical region surrounding the dialysis probe. Previous studies determined the size of the cylindrical brain region was approximately 3 mm deep and 2–3 mm in diameter (Lindefors et al., 1989). A solution of 2-methyl-carbamazepine (5 pmol/μl) in 0.9% sodium chloride perfused the jugular microdialysis probe. A Harvard pump produced perfusion rates for both microdialysis probes of 2 μl/min. Pharmacokinetic studies occurred after animals received i.p. injections of carbamazepine (12 mg/kg) administered as an inclusion complex. The carbamazepine inclusion complex was composed of carbamazepine and 2-hydroxypropyl-β-cyclodextrin (Molecusol, Pharmatec, Alachua, FL, USA). The sampling times for microdialysis probe effluent (dialysate) were every 20 min for 1 h before the dose, then every 20 min for 12 h after the dose. Analysis with HPLC (high performance liquid chromatography) measured carbamazepine, carbamazepine-10,11-epoxide, and 2-methyl-carbamazepine. The 2-methyl-carbamazepine served as an internal standard for quantitation of carbamazepine and carbamazepine-10,11-epoxide (van Belle et al., 1995b). In addition, 2-methyl-carbamazepine assay permitted determination of the efficiency (recovery) of microdialysis probes.

Data from previous studies provided the rationale for the choice of the i.p. carbamazepine dose. In GEPR-9s, the

ED_{50} for i.p. carbamazepine against audiogenic seizures was 3 mg/kg (Dailey and Jobe, 1985). For Sprague–Dawley rats, the ED_{50} against electroshock seizures was 7.5 mg/kg carbamazepine i.p. (Dailey et al., 1997b). In GEPR-3s, the ED_{50} against audiogenic seizures was 25 mg/kg i.p. (Dailey and Jobe, 1985). The carbamazepine dose, 12 mg/kg i.p., in the present experiment represented a mid-range effective dose for the different strains of rats employed. Dose-limiting toxicity prevented the use of higher doses. A carbamazepine dose four times ED_{50} was fatal to GEPR-3 animals (Dailey et al., 1997a). Carbamazepine pharmacokinetic parameters are known to be dose-dependent within the range of doses 5–20 mg/kg (Remmel et al., 1990). Therefore, a single-dose experimental design was employed to test the hypothesis regarding strain-specific pharmacokinetic or pharmacodynamic differences.

2.3. Pharmacodynamic procedures

GEPR-3s and GEPR-9s were prepared for microdialysis studies as previously described (Yan et al., 1992; Dailey et al., 1997a,b). To place guide cannulae, animals were anesthetized and the guides were placed over the hippocampus without penetrating the dura. The coordinates relative to bregma were anteroposterior—5.2 mm, lateral 5.0 mm (Paxinos and Watson, 1986). Animals were allowed to recover for 5 days prior to microdialysis experiments. On the experimental day, the microdialysis probe was inserted into the guide and directed to the hippocampus with the tip 7.2 mm below the dura. Rats were then placed into Plexiglas™ chambers and allowed to move freely. Probes were perfused at a constant flow rate of 1.6 μl/min with artificial cerebrospinal fluid. After discarding the first 60 min of dialysate, which typically contained large concentrations of serotonin, dialysate was collected to confirm stable basal release of serotonin. At least three basal serotonin measurements occurred prior to introduction of carbamazepine. After starting the continuous focal infusion of each carbamazepine concentration, dialysate samples were collected every 20 min for 80 min.

Various carbamazepine concentrations were administered through the microdialysis probe as continuous focal infusions to the hippocampus. Because of its limited solubility in artificial cerebrospinal fluid, carbamazepine was administered as the inclusion complex (carbamazepine plus 2-hydroxypropyl-β-cyclodextrin) as previously described (Dailey et al., 1997b). In vivo dialysis probe recovery for carbamazepine was 32.07 ± 1.26% (mean ± S.E.M.) (Dailey et al., 1997b). Carbamazepine concentrations dissolved in dialysate were 50, 250 and 1250 μM. When corrected for probe recovery, the estimated concentrations delivered to brain tissue were 16, 64 and 400 μM, respectively. These concentrations compare favorably with the concentrations required to release serotonin from hippocampal slices in vitro (Dailey et al., 1998).

Previous studies provided the rationale for the choice of carbamazepine concentrations in the microdialysis perfusate. Systemic administration of four times the ED_{50} carbamazepine dose produced a hippocampal extracellular carbamazepine concentration of 11–12 μM and increased extracellular serotonin approximately fourfold (Dailey et al., 1997b). After focal administration, a similar increase in extracellular serotonin is produced by 400 μM carbamazepine (Dailey et al., 1997b). The carbamazepine concentrations in the microdialysis perfusate were equipotent with the systemic dose of carbamazepine used in the pharmacokinetic experiments.

2.4. Histological procedure

At the close of the experiments, rats were decapitated and their brains were immersion-fixed overnight in buffered 4% paraformaldehyde. Coronal sections 40-μm-thick were cut on a freezing microtome, stained with neutral red and analyzed in a light microscope to verify dialysis probe location. Data from brains in which the probes were improperly placed were not analyzed. Autopsy confirmed jugular probe placement. If autopsy showed the jugular microdialysis probe did not extend beyond the jugular catheter, then data from the animal were not included in the pharmacokinetic analyses.

2.5. Chromatographic conditions

The assay employed for carbamazepine analogues was published previously (Scheyer et al., 1994). In summary, perfusate and dialysate samples were injected onto the HPLC system (Waters, Milford, MA, USA) with ultraviolet detection. Separation occurred on an Econosphere C18, 3 μm column (Alltech, Deerfield, IL, USA). The mobile phase contained water, acetonitrile, and methanol (60/23/17 volume/volume). The pump ran in isocratic mode at 0.8 ml/min at constant temperature (40°C). Standards were 0.5 pmol/μl solutions of carbamazepine, 2-methyl-carbamazepine, and carbamazepine-10,11-epoxide. Peaks were detected at 210 nm wavelength. Chromatograms were integrated and analyzed using a computerized data acquisition system (EZ Chrom, Scientific Software, San Ramon, CA, USA).

For serotonin analysis, dialysates were injected directly into the HPLC with electrochemical detection as previously described (Yan et al., 1992). The HPLC system was an ESA (Bedford, MA, USA) solvent delivery system (model 580) consisting of a dual piston pump, an ESA HR-80 column (3 μm, ODS, 80 × 4.6 mm) and a Coulochem II electrochemical detector (Model 5200A). Detector output was recorded on a Shimadzu C-R6A integrator. The mobile phase contained 75 mM Na_2HPO_4, 1.5 mM sodium dodecyl sulfate, 20 μM EDTA, 100 μl/l triethylamine (pH 5.6 with H_3PO_4), 12% methanol and 12% acetonitrile and was delivered through the system at 1 ml/min.

2.6. Protein-bound carbamazepine concentration procedure

Animals received i.p. injections of carbamazepine 12 mg/kg. After 2 h, the halothane-anesthetized animal underwent cardiac puncture for collection of 3 ml blood into a heparin-coated syringe. Centrifugation yielded plasma used to measure total carbamazepine and protein-bound carbamazepine.

To obtain total carbamazepine concentrations, plasma (500 μl) was placed in a glass test tube with 2-methyl-carbamazepine internal standard (5 μl of a 500 pmol/μl solution). The sample was extracted by adding phosphate buffer (1 ml of 0.1 M) and chloroform (5 ml, HPLC grade). After centrifugation, the chloroform layer was transferred to a clean tube and evaporated to dryness. The samples were reconstituted with mobile phase (200 μl) before injection into the HPLC system (Sawchuk and Cartier, 1982).

To obtain protein-bound carbamazepine concentrations, plasma (500 μl) was placed in a Tube-O-DIALYZER™ (40,000 Da cut-off, Research Products International, Mount Prospect, IL, USA) with 2-methyl-carbamazepine internal standard (5 μl of a 500 pmol/μl solution). The plasma plus 2-methyl-carbamazepine dialyzed for 5 h at room temperature against Ringer's solution. After dialysis, the plasma plus 2-methyl-carbamazepine sample was transferred to a glass test tube. Then, extraction, centrifugation, evaporation, reconstitution, and injection occurred as described above for total carbamazepine analysis.

A standard curve was created and extracted in the same manner. Standards were also analyzed with each extraction group. Percentage protein-bound carbamazepine was calculated by dividing bound carbamazepine concentration by total carbamazepine concentration.

2.7. Analysis of data

Concentrations of carbamazepine and carbamazepine-10,11-epoxide were calculated from the relative loss (RL) of the internal standard, 2-methyl-carbamazepine, using the formula: $RL = (C_p - C_d)/C_p$ where C_p = perfusate concentration and C_d = dialysate concentration (van Belle et al., 1993). Inspection of carbamazepine concentration-vs.-time plots for each animal revealed the following pharmacokinetic parameters: maximum concentration (C_{max}), time to peak concentration (t_{max}). Values for terminal elimination rate constant (k_e) and elimination half-life ($t_{1/2}$) were calculated by least squares linear regression analysis of the terminal elimination portion of the log concentration-vs.-time curves. The values for area under the concentration-vs.-time curve (AUC) from time zero to last measurement were calculated by the trapezoidal method. The calculated area from time of last measurement until infinity was C_n/k_e (C_n was the last measured concentration). Pharmacokinetic parameter values for groups were compared using analysis of variance with Tukey's test for multiple comparisons. If data did not satisfy assumptions of a normal distribution (Kolmogorov–Smirnov test), then group comparisons employed the Kruskal–Wallis test with Dunn's test for multiple comparisons. The P value less than 0.05 was considered significant.

The measured pharmacodynamic effect was the change from basal serotonin concentration induced by focal continuous infusion of carbamazepine. Plots of effect-vs.-time expressed the percentage of mean basal serotonin concentration obtained in each rat's hippocampus. Area under the effect-vs.-time curve (AUEC) from time zero to 80 min was calculated by the trapezoidal rule. Statistical comparisons of AUEC values between groups of rats and between concentrations of carbamazepine employed the Kruskal–Wallis test.

3. Results

3.1. Pharmacokinetic studies

Experiments revealed significant differences between rat groups in carbamazepine area under the concentration-vs.-time curve (AUC). After receiving single i.p. doses of carbamazepine (12 mg/kg), three rat groups had their unbound carbamazepine concentrations measured in the brain and plasma by microdialysis (see Fig. 1). AUC values in GEPR-9 brains significantly exceeded values in control rat brains and GEPR-3 brains (see Table 1). The rank order of AUC values in brain and plasma for GEPR-9, control, and GEPR-3 parallels the rank order in carbamazepine anticonvulsive potency in these three groups of animals.

The differences between AUC values for GEPR-9, GEPR-3, and control animals were consistent with differences in maximum concentration (C_{max}). When comparing maximum brain carbamazepine concentrations between groups, there was a significant difference between GEPR-9 and GEPR-3 animals (see Table 1). The rank order of maximum brain carbamazepine concentrations for GEPR-9, control, and GEPR-3 parallels the rank order in carbamazepine potency in these three groups of animals.

The times to maximum carbamazepine brain concentration (t_{max}) were similar between groups and comparable to previous published data (van Belle et al., 1995a). In the present study, differences in AUC between groups were not explained by differences in plasma or brain t_{max} (Table 1).

The terminal elimination portion of the carbamazepine log concentration-vs.-time curves revealed log–linear decline as previously reported (Remmel et al., 1990; van Belle et al., 1995a). The terminal elimination half-lives for brain and plasma in Table 1 are also comparable to

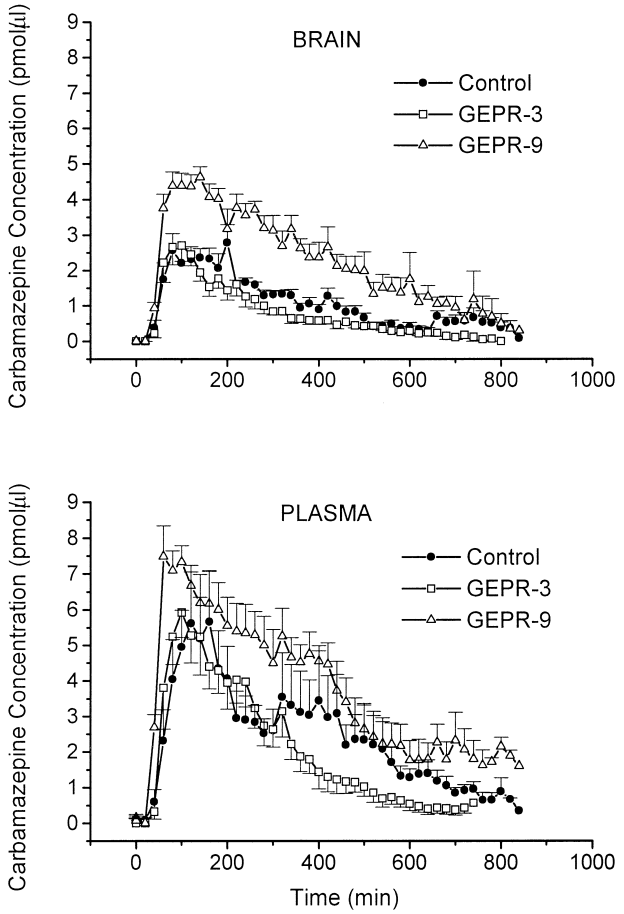

Fig. 1. Pharmacokinetic plots in various rat strains after single-dose i.p. injections of carbamazepine 12 mg/kg show concentration vs. time. Unbound concentrations of carbamazepine were measured in the brain and plasma by microdialysis. GEPR-3 and GEPR-9 are distinct strains of genetically epilepsy-prone rats. Each symbol shows the mean ± S.E.M. In the upper panel (brain concentrations), the number of animals in each group were 8, 8, and 9 for controls, GEPR-3s, and GEPR-9s. In the lower panel (plasma concentrations), the number of animals in each group were 6, 7, and 6 for controls, GEPR-3s, and GEPR-9s.

previous data (van Belle et al., 1995a). In the present study, differences in AUC between groups were not explained by differences in terminal elimination half-life (Table 1).

Measurements of plasma protein binding did not explicate the AUC differences between groups of rats. Mean plasma protein binding in control, GEPR-3 and GEPR-9 animals (mean ± S.E.M.) were 61% ± 5%, 62% ± 7%, and 67% ± 6%, respectively (sample sizes, *n*, were 9, 9, and 10, respectively). These values are similar to previously published values (Remmel et al., 1990).

Estimates of carbamazepine brain transport derived from the ratio of brain AUC to plasma AUC. These mean ratios were 0.55, 0.51, and 0.54 for Sprague–Dawley rats, GEPR-3s and GEPR-9s, respectively. The similarity in carbamazepine brain transport ratios suggests differential transport across the blood-brain barrier did not explain the

Table 1
Carbamazepine pharmacokinetic parameters (mean ± S.E.M.) in different rat strains following single i.p. doses of carbamazepine 12 mg/kg

Pharmacokinetic parameter (units), number of rats	GEPR-9	Control	GEPR-3
Plasma AUC (pmol min/μl), $n = 6$	3617 ± 517[a]	2286 ± 508	1551 ± 305
Brain AUC (pmol min/μl), $n = 8$	1899 ± 219[a,b]	886 ± 40	658 ± 77
Plasma C_{max} (pmol/μl), $n = 6$	8 ± 1	6 ± 1	7 ± 1
Brain C_{max} (pmol/μl), $n = 8$	5 ± 0.3[a]	4 ± 0.4	3 ± 0.4
Plasma t_{max} (min), $n = 6$	120 ± 40	143 ± 31	120 ± 14
Brain t_{max} (min), $n = 7$	126 ± 9	129 ± 21	89 ± 9
Plasma half-life (min), $n = 6$	283 ± 51	257 ± 47	138 ± 18
Brain half-life (min), $n = 8$	197 ± 28	205 ± 28	150 ± 19

AUC = Area under concentration-vs.-time curve.
C_{max} = maximum concentration.
t_{max} = time to achieve maximum concentration.
GEPR = genetically epilepsy-prone rat.
a = GEPR-9 vs. GEPR-3, $P < 0.05$.
b = GEPR-9 vs. Control, $P < 0.05$.

differences in pharmacodynamic response between rat strains after systemic carbamazepine administration.

Carbamazepine-10,11-epoxide is the active metabolite of carbamazepine. Concentration-vs.-time plots for carbamazepine-10,11-epoxide revealed overlapping curves for GEPR-9s, GEPR-3s and controls. The differences in carbamazepine potency between rat groups were not attributable to strain-specific differences in carbamazepine-10,11-epoxide pharmacokinetics.

The mean elimination half-life for brain concentrations of carbamazepine-10,11-epoxide ranged from 139 to 225 min and confirmed previously reported values (van Belle et al., 1995a).

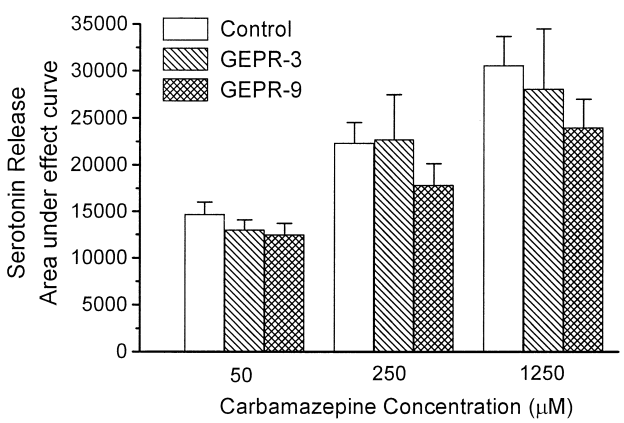

Fig. 2. Serotonin release (*y*-axis) compares with continuous focal intracerebral perfusions of various carbamazepine concentrations (*x*-axis). The pharmacodynamic effect is the percentage change in mean basal serotonin concentration measured in rat hippocampus by microdialysis. The areas under the effect-vs.-time curve (AUEC) appear on the *y*-axis for three rat strains (control, GEPR-3, GEPR-9). The units for AUEC are percent change multiplied by minutes. Rats received carbamazepine concentrations as continuous infusions via microdialysis probes. Each bar represents mean ± S.E.M. for eight rats.

3.2. Pharmacodynamic studies

In the next set of experiments, groups of rats received focal continuous infusions of carbamazepine into the hippocampus via microdialysis probes. Perfusate carbamazepine concentrations were 50, 250, 1250 μM. The pharmacodynamic response was change from basal intracerebral serotonin concentration. Fig. 2 plots values for the area-under-the-effect-vs.-time curves (AUEC) at each concentration of infused carbamazepine. Each group of animals (control, GEPR-3, GEPR-9) demonstrated a significant concentration–response relationship to carbamazepine focal perfusion. At each concentration level, there was no significant AUEC difference between groups.

4. Discussion

The present study attempted to answer the following questions about the mechanism of carbamazepine action in controlling seizures. When comparing GEPR-9, GEPR-3 and control rats, are the differences in carbamazepine anticonvulsant potency due to pharmacokinetics or pharmacodynamics? Similarly, are the carbamazepine-induced differences in serotonin release due to pharmacokinetics or pharmacodynamics?

Previous studies show GEPR-9s respond to the lowest doses of carbamazepine while Sprague–Dawley rats are intermediate in responsiveness and GEPR-3s are the least sensitive to the anticonvulsant effects of carbamazepine (Dailey and Jobe, 1985; Dailey et al., 1997a,b). The present results suggest at least part of the difference in responsiveness among the three strains is pharmacokinetic in nature. After a 12 mg/kg dose of carbamazepine, the AUC for carbamazepine in brain extracellular fluid and in plasma is largest in GEPR-9s, intermediate in Sprague–Dawley rats and lowest in GEPR-3s.

The results in Fig. 2 suggest the differences in carbamazepine-induced serotonin release may also be due to pharmacokinetics rather than pharmacodynamics. When various concentrations are administered directly to the brain, carbamazepine appears to be equipotent between GEPR-9, GEPR-3, and control rats. When carbamazepine is administered to the peritoneum, there are pharmacokinetic differences in carbamazepine AUC between groups of rats. Strain-specific pharmacokinetic differences correlate with pharmacodynamic differences in serotonin release after systemic carbamazepine administration. For example, GEPR-9 brains have the highest AUC and GEPR-9 animals have the greatest serotonin release from i.p. injection of carbamazepine (Dailey et al., 1997a). These data suggest pharmacokinetic differences between GEPR-9, GEPR-3, and control rats explain the differences in serotonin release.

As noted above, each of the three seizure models (electroshock, GEPR-3, GEPR-9) employed in these studies experience anticonvulsant effects from carbamazepine. However, carbamazepine has many cellular actions that could contribute to its anticonvulsant activity. It is possible that different cellular effects could mediate carbamazepine's anticonvulsant action in the three models. If this were the case, pharmacodynamic differences among the three strains could explain the dose–response characteristics of carbamazepine. It seems more likely that release of serotonin by carbamazepine contributes importantly to the anticonvulsant mechanism of this drug in each of the strains. In the electroshock model, drugs which decrease serotonin release or lesions which deplete serotonin lower the seizure threshold and increase seizure severity (see Browning, 1987 for a review of the electroshock model). Conversely, drugs that release serotonin increase the electroshock seizure threshold and decrease the intensity of these seizures. Similarly, in GEPRs, drugs which decrease serotonin release or lesions that deplete serotonin intensify seizures and drugs which release serotonin are anticonvulsant (Jobe et al., 1986; Reigel et al., 1986; Dailey et al., 1989). It should be noted that depletion of serotonin greatly decreases but does not abolish the anticonvulsant effectiveness of carbamazepine in GEPRs (Yan et al., 1992; Dailey et al., 1996). Thus, it seems probable that other cellular actions of carbamazepine such as the well-documented effect on sodium channels and repetitive firing of action potentials (Macdonald and McLean, 1986; McLean and Macdonald, 1986) contribute to its anticonvulsant activity in the GEPR and in other animal models.

Since an integral part of microdialysis involves movement of solute across a semipermeable membrane with a molecular weight cutoff (6000 Da) well below the weight of proteins, this procedure measures concentrations of drugs unbound to plasma proteins. Thus, the observed AUC differences (Table 1) theoretically could be the result of differences in binding to plasma proteins among the three strains. However, mean plasma protein binding was measured in the three rat strains and there was no difference among the strains in these measurements.

What explains the pharmacokinetic differences observed between groups of rats in the present study? Differences in absorption time, terminal elimination time, and plasma protein binding do not answer the question. Inhibition of cytochrome P450 metabolism would be a potential explanation since inhibitors are known to increase carbamazepine half-life (van Belle et al., 1995a). However, the present study design controlled for differential effects on carbamazepine metabolism by inhibitors or inducers of cytochrome P450. The present study design did not control for inherited or intrinsic differences between rat strains in their cytochrome P450 metabolism of carbamazepine. The pattern of pharmacokinetic difference between GEPR-9s and controls or GEPR-3s, (increased AUC, increased C_{max}, unchanged t_{max}, unchanged terminal elimination) is similar to the pattern demonstrated when humans receive oral

carbamazepine concomitantly with oral inhibitors of cytochrome P4503A4 (Wong et al., 1983; Garg et al., 1998). If GEPR-9s had lower presystemic clearance of carbamazepine, they might be expected to have higher values for AUC and C_{max}. Future studies should address the hypothesis of strain-specific differences in presystemic clearance.

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