ELSEVIER

European Journal of Pharmacology 328 (1997) 153–162

ejp

Carbamazepine increases extracellular serotonin concentration: lack of antagonism by tetrodotoxin or zero Ca^{2+}

John W. Dailey *, Maarten E.A. Reith, Qing-Shan Yan, Ming-Ya Li, Phillip C. Jobe

Department of Biomedical and Therapeutic Sciences, University of Illinois College of Medicine at Peoria, P.O. Box 1649, Peoria, IL 61656, USA

Received 19 December 1996; revised 2 April 1997; accepted 8 April 1997

Abstract

Carbamazepine administration causes large increases in extracellular serotonin concentration and dose-related anticonvulsant effects in genetically epilepsy-prone rats (GEPRs). In order to determine the generality of the effect on serotonin, we determined the anticonvulsant ED_{50} for carbamazepine against maximal electroshock seizures in outbred, non-epileptic Sprague-Dawley rats. We then administered anticonvulsant carbamazepine doses to Sprague-Dawley rats and observed extracellular serotonin concentration in hippocampi by way of microdialysis. We found that administration of carbamazepine, either systemically or through the dialysis probe, resulted in significant and dose-related increases in extracellular serotonin concentration. Basal serotonin release was decreased by tetrodotoxin administration through the dialysis probe. Tetrodotoxin administration through the dialysis probe did not decrease the effect of systemically or focally administered carbamazepine on extracellular serotonin concentration. Similarly, elimination of Ca^{2+} from the dialysate did not alter the release of serotonin caused by carbamazepine. These findings suggest that the serotonin releasing effect of carbamazepine does not take place by exocytosis and does not require action potentials in the brain area in which the release takes place. Further they suggest that the effect is mediated by an action of carbamazepine directly on serotonergic nerve terminals.

Keywords: Carbamazepine; 5-HT (5-hydroxytryptamine, serotonin); Microdialysis; Hippocampus; Ca^{2+}; Electroshock seizure; Tetrodotoxin; (Rat)

1. Introduction

Substantial data show that central nervous system serotonin plays an anticonvulsant role in most mammalian seizure models. Increasing serotonin release causes an anticonvulsant effect in maximal electroshock seizures in rats and mice (Buterbaugh, 1977; Browning, 1987; Browning et al., 1978), limbic and thalamic seizures in cats (Wada et al., 1992a,b), sensory induced seizures in El mice (Hiramatsu et al., 1987), audiogenic seizures in genetically epilepsy-prone rats (GEPRs) (Jobe et al., 1973b; Dailey et al., 1989, 1992a,b; Jobe et al., 1982), audiogenic seizures in DBA/2J mice (Sparks and Buckholtz, 1985) and in seizures induced by focal injection of bicuculine into area tempestis of the deep prepiriform cortex of rats (Pasini et al., 1992; Prendiville and Gale, 1993).

Administration of anticonvulsant doses of carbamazepine to GEPRs causes a 7–9-fold increase in hippocampal extracellular serotonin as measured by microdialysis (Yan et al., 1992). These doses had no measurable effect on extracellular norepinephrine. CNS (central nervous system) monoamines are of etiologic importance in the seizure predisposition that characterizes GEPRs. These animals have abnormally low CNS levels of serotonin (Dailey et al., 1992a; Jobe et al., 1986, 1982) and norepinephrine (Dailey et al., 1991; Jobe et al., 1982, 1986). These abnormalities exist in GEPRs that have experienced sound-induced seizures (Jobe et al., 1986) as well as in GEPRs that are naive to seizures (Dailey et al., 1991, 1992a). Studies with pharmacologic agents have indicated that drugs which further decrease the already low concentration of norepinephrine or serotonin cause intensification of seizures in GEPRs, while agents which enhance serotonergic or noradrenergic transmission are anticonvulsant in these animals (Dailey et al., 1989; Jobe et al., 1973a,b; Laird et al., 1984). Depletion of serotonin in GEPRs by inhibition of serotonin synthesis abolishes much of the anticonvulsant effectiveness of carbamazepine in these animals (Yan et al., 1992).

Because GEPRs are abnormal with respect to their CNS neurochemistry and their response to seizure provoking stimuli, there is some possibility that this effect of carba-

* Corresponding author. Tel.: (1-309) 671-8528; Fax: (1-309) 671-8403; e-mail: jwd@uic.edu

mazepine on extracellular serotonin occurs only in GEPRs. In order to determine if anticonvulsant doses of carbamazepine have a similar effect on serotonin release in non-epileptic animals, we determined the ED_{50} for carbamazepine against supramaximal electroshock seizures in Sprague-Dawley rats and in a second group of Sprague-Dawley rats, we administered anticonvulsant doses of carbamazepine while analyzing hippocampal extracellular serotonin concentration through the use of microdialysis coupled with high performance liquid chromatography (HPLC). Further, we administered carbamazepine through the microdialysis probe while monitoring extracellular serotonin content.

2. Materials and methods

2.1. Animals

Animals used in these studies were outbred, non-epileptic Sprague-Dawley rats purchased from Harlan Sprague-Dawley (Indianapolis, IN, USA). This is the strain of rats from which GEPRs were originally derived (Reigel et al., 1986). The Sprague-Dawley rats used in this study (200–250 g females) were housed at 21 ± 3°C with 40–60% relative humidity and were maintained under 12 h light/12 h dark conditions with ad libitum access to food and water.

2.2. Drugs

Carbamazepine (Sigma, St. Louis, MO, USA) was suspended in methylcellulose (Sigma) prior to intraperitoneal (i.p.) injections. Because of its limited solubility in artificial cerebrospinal fluid (CSF), when carbamazepine was administered through the dialysis probe, a carbamazepine complex (PR-320, Pharmatec) was dissolved in artificial CSF. The carbamazepine inclusion complex was composed of carbamazepine and Molecusol (2-hydroxypropyl-β-cyclodextrin, Pharmatec). Tetrodotoxin (Sigma) was dissolved in artificial cerebrospinal fluid and administered through the dialysis probe.

2.3. Maximal electroshock seizures

The carbamazepine ED_{50} dose (effective dose in 50% of animals) against maximal electroshock seizures in rats is reported to be 7–10 mg/kg (Faigle and Feldman, 1989). We confirmed the ED_{50} for carbamazepine against maximal electroshock seizures in outbred Sprague-Dawley rats using a Wahlquist stimulator (Swinyard, 1972; Jobe et al., 1992). The electrical stimulus (150 mA, 60 Hz, 0.2 s) was administered 1 h after i.p. carbamazepine administration. Groups of 10 rats received one of three doses of carbamazepine i.p. (5, 9 or 16 mg/kg). Abolition of tonic hindlimb extension was the endpoint for an anticonvulsant effect (Swinyard, 1972; Jobe et al., 1992). Since not all Sprague-Dawley rats have the capacity to experience tonic hindlimb extension, all rats used in the electroshock experiments were tested one day prior to drug administration to ascertain that they did in fact exhibit hindlimb extension. Animals that did not exhibit hindlimb extension were eliminated from experimentation. The previously determined time of maximal anticonvulsant effect for this carbamazepine dosage form in our laboratory had been 2–3 h after i.p. drug administration. However, a misunderstanding caused the experiment to be carried out 1 h after the drug administration. Because the ED_{50} calculated from this experiment (7.5 mg/kg) was very close to the literature value we did not repeat the dose–response study.

2.4. Microdialysis

Loop type microdialysis probes similar to those used in earlier studies (Yan et al., 1992) were constructed of cellulose acetate dialysis fibers (I.D. 215 ± 15 μm, molecular weight cutoff = 6000; Spectrum Medical Industries, Los Angeles, CA, USA) affixed to fused silica tubing (Polymicro Technologies). The active dialysis area was 6 mm in length with the dialysis probes folded in the middle such that the brain area dialyzed was 3 mm in depth.

For placement of guide cannulae, animals were anesthetized using a combination of sodium pentobarbital (20 mg/kg, i.p.) and 5% halothane in oxygen. While anesthetized, the animals were placed in a Kopf stereotaxic frame and a 22-gauge guide cannula was affixed to the animal's skull with dental acrylic and Teflon machine screws. These guides were placed over the hippocampus without penetrating the dura. The coordinates relative to bregma were: anteroposterior −5.2 mm, L 5.0 mm (Paxinos and Watson, 1986). Five days were allowed for the animals to recover from surgery before microdialysis experiments were carried out.

For microdialysis experiments, a dialysis probe was inserted into the guide and directed to the hippocampus with the tip 7.2 mm below the dura. Rats were then placed individually into Plexiglas chambers and allowed to move freely about. Probes were perfused at a constant flow rate of 1.6 μl per minute with artificial cerebrospinal fluid which contained (in mM) Na^+ (150), K^+ (3.0), Ca^{2+} (1.2), Mg^{2+} (0.8), Cl^- (155). After discarding the first 60 min of dialysate, which typically contained large concentrations of transmitter compounds, stable basal release dialysate collections were obtained before drugs were administered. For all experiments, at least three basal release measurements were made prior to introduction of a drug.

In experiments in which tetrodotoxin was administered through the dialysis probe after systemic carbamazepine administration, a control period (30 min) in which basal serotonin release was measured preceded the addition of tetrodotoxin (1 μM or 10 μM) to the artificial CSF. After 40 min of perfusion with artificial CSF containing the

tetrodotoxin, carbamazepine was administered i.p. and dialysis samples were collected over the next 4.5 h.

In one experiment, carbamazepine was administered i.p. and the concentration of carbamazepine, its anticonvulsant metabolite, carbamazepine-10,11-epoxide (Bourgeois and Wad, 1984) (carbamazepine-epoxide, gift from Ciba-Geigy) and serotonin were measured in dialysates collected from hippocampus.

In several experiments, carbamazepine (as an inclusion complex with Molecusol) was administered through the microdialysis probe and serotonin was measured as the dialysates were collected. In these experiments, carbamazepine-induced serotonin release was measured over 60 min and the dialysate was switched to one containing the carbamazepine inclusion complex plus either tetrodotoxin (1.5 μM in artificial CSF) or zero calcium artificial CSF. The zero calcium artificial CSF contained (in mM) Na^+ (150), K^+ (3.0), Mg^{2+} (2.2), Cl^- (155.6) and EGTA (0.4).

2.5. Analytical and histological procedures

Following systemic administration of carbamazepine, the concentration of carbamazepine, its anticonvulsant metabolite carbamazepine-epoxide and serotonin were measured in dialysates from hippocampus. Dialysates were collected in a CMA refrigerated fraction collector (CMA, Stockholm, Sweden). Approximately half of each dialysate was used for immediate serotonin analysis while the remainder was prepared for analysis of carbamazepine and carbamazepine-epoxide according to the method of Scheyer et al. (1994). For this analysis, we used a Waters HPLC system (Milford, MA, USA) with UV detection. The Waters HPLC system consisted of a 490 UV detector, 616 pump, 600S controller, and a 717 plus autosampler. Separation was performed on an Econosphere C18, 3 μm (100 mm × 4.6 mm I.D.) (Alltech, Deerfield, IL, USA) column. Integration was carried out using an EZChrom chromatographic software system (Scientific Software, San Ramon, CA, USA). The pump was run in an isocratic mode at a flow rate of 0.8 ml/min with a mobile phase of water/acetonitrile/methanol (60:23:17, v/v) with the column at a constant temperature of 40°C. Peaks were detected at a wavelength of 210 nm with a 20 μl injection volume. The limit of detection for carbamazepine and carbamazepine-epoxide achieved was 0.2 μg/ml.

For analysis of serotonin, dialysates were directly injected into a HPLC with electrochemical detection without further preparation as was described previously (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. Detector output was recorded on a Shimadzu C-R6A integrator. Integration was carried out using an EZChrom chromatographic software system (Scientific Software). The mobile phase consisted of 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 pumped through the system at 1 ml/min. For experiments in which drugs were administered through the dialysis probe, serotonin was measured as the dialysates were collected.

Following completion of dialysis, animals 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. The location of the dialysis probe was verified for each brain. Data from brains in which the probes were not properly placed were not included in the present study.

2.6. Probe recovery

In order accurately to measure the concentration of carbamazepine in the extracellular fluid dialyzed from hippocampus, in vivo probe recovery was determined according to the 'reverse dialysis' procedure (Le Quellec et al., 1995). Since solute diffusion occurs in both directions across the dialysis membrane, loss of solute from the dialysate occurs at the same rate as recovery of solute into the dialysate. Thus, carbamazepine (4 μM) was dissolved in artificial CSF and administered to the animal through a dialysis probe placed in the hippocampus. Loss of carbamazepine across the dialysis membrane averaged 35.88 ± 1.64% for the 4 probes evaluated. In order to facilitate its dissolution in artificial CSF, carbamazepine (4 μM) was first dissolved in dimethyl sulfoxide (DMSO) and diluted to a final DMSO concentration of 0.002%.

In experiments in which carbamazepine was administered through the dialysis probe, it was complexed with β-cyclodextrin. Loss of carbamazepine complexed with β-cyclodextrin was determined in a manner analogous to that described for carbamazepine above. Carbamazepine complexed with β-cyclodextrin was dissolved in artificial CSF at a concentration of 4 μM. Loss of carbamazepine from the dialysate averaged 32.07 ± 1.26% (mean ± S.E.M.) for the 4 probes evaluated.

2.7. Statistical analysis

Changes in concentrations of serotonin induced by administration of carbamazepine were expressed as percentage of the mean basal output obtained in each individual rat. These were compared statistically with the control period values by use of analysis of variance (ANOVA) for repeated measures. The dose–response relationship for the carbamazepine anticonvulsant effect was evaluated according to the method of Litchfield and Wilcoxon (1949). Area under the concentration-time curve evaluations were made using the trapezoid rule. The Pearson product moment correlation was used to determine whether there was a significant correlation between increasing dose and increasing area under the concentration-time curves. The

statistical tests were actually carried out by use of Sigma-Stat.

3. Results

3.1. Carbamazepine ED_{50} against maximal electroshock seizures

The carbamazepine ED_{50} dose (effective dose in 50% of animals) against maximal electroshock seizures was confirmed by experiment in our laboratory. The ED_{50} with confidence interval we obtained in 30 outbred, female Sprague-Dawley rats was 7.5 (5.2–11.0) mg/kg i.p.

3.2. Dose-related increase in extracellular serotonin produced by carbamazepine

Fig. 1a shows the effect of three different i.p. doses of carbamazepine on extracellular serotonin (expressed as percent of baseline) in outbred Sprague-Dawley rats. Basal serotonin release (mean ± S.E.M.) was 13.97 ± 1.33, 13.00 ± 1.50 and 10.44 ± 1.44 fmol/sample for the 8, 16 and 32 mg/kg groups, respectively. There were no statistically significant differences in basal serotonin release among the groups. Fig. 1b shows the area under the concentration–time curve for the three doses of carbamazepine used in this experiment. Area under the concentration-time curve estimates the total serotonin released by the carbamazepine dose administered. Prior to drug administration, the mean serotonin concentrations in hippocampal extracellular fluid in the three groups of rats were not significantly different from each other. The 16 and 32 mg/kg doses of carbamazepine caused significant increases in extracellular serotonin from hippocampi of these animals. The maximal increases in extracellular serotonin occurred between 3 and 4 h after drug administration, depending on the dose. The carbamazepine-induced elevations in extracellular serotonin appeared to be sustained over at least a 5 h period. The carbamazepine doses used

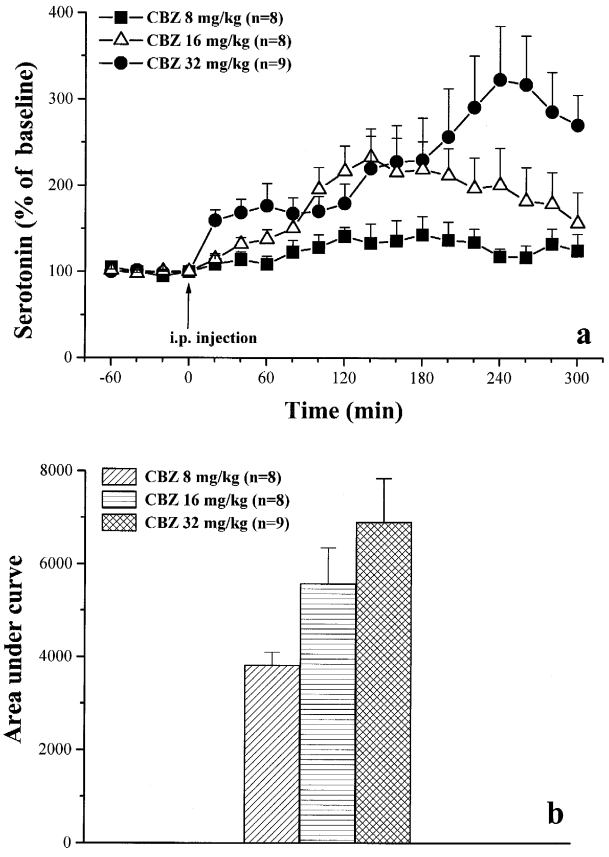

Fig. 1. (a) The increase in extracellular serotonin (mean ± S.E.M.) from hippocampus of Sprague-Dawley rats following i.p. carbamazepine (CBZ) administration (at the arrow). The 16 and 32 mg/kg doses produced statistically significant ($P < 0.001$ in each case, ANOVA) treatment effects. (b) The area under the concentration–time curve (mean ± S.E.M.) for the three curves depicted in panel a. Doses and number of animals (n) are shown in panel b. $P = 0.0083$ (Pearson product moment) for the regression of dose vs. area under the concentration–time curve in panel b.

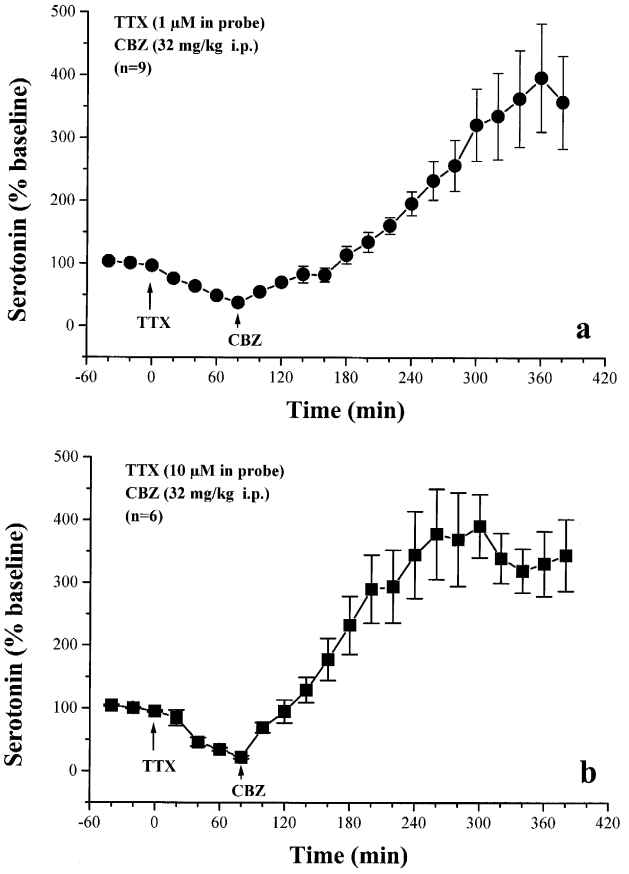

Fig. 2. Effect of 1 μM (a) or 10 μM (b) tetrodotoxin on increases in extracellular serotonin concentration (mean ± S.E.M.) dialyzed from the hippocampus of Sprague-Dawley rats. Tetrodotoxin was perfused through the dialysis probe from time zero until the end of the experiment. Carbamazepine (CBZ, 32 mg/kg) was given i.p. at the time indicated. Doses and number of animals (n) are shown in the figure. Carbamazepine produced a statistically significant treatment effect, $P < 0.001$ (ANOVA).

in this experiment approximated the ED_{50}, twice the ED_{50} and four times the ED_{50} for carbamazepine against maximal electroshock seizures.

3.3. Effect of tetrodotoxin on extracellular serotonin in carbamazepine-treated rats

Tetrodotoxin blocks sodium channels and prevents action potentials. Infusion of 1 μM tetrodotoxin (in artificial cerebrospinal fluid) through the dialysis probe caused approximately a 50% reduction in basal serotonin release (Fig. 2a). Basal serotonin release in this group was 13.10 ± 1.81 fmol/sample. Administration of carbamazepine i.p. caused a significant and sustained increase in extracellular fluid serotonin concentration even though the infusion of 1 μM tetrodotoxin continued for the duration of the experiment. Fig. 2b shows the effect of 10 μM tetrodotoxin on basal serotonin release and serotonin release caused by i.p. carbamazepine administration. The 10 μM tetrodotoxin infusion through the dialysis probe decreased basal serotonin release which was 7.44 ± 0.81 fmol/sample by more than 75%, yet this high tetrodotoxin concentration had no apparent effect on the serotonin release caused by carbamazepine. Indeed, the maximal serotonin release caused by the 32 mg/kg dose of carbamazepine is actually slightly higher in animals treated with either of the tetrodotoxin concentrations than it is in the animals dialyzed only with artificial CSF. However, the areas under the concentration–time curves for the animals receiving tetrodotoxin and those that did not are not significantly different. These data demonstrate that neither the 1 μM nor the 10 μM concentration of tetrodotoxin decreased the release of serotonin caused by carbamazepine.

3.4. Carbamazepine, carbamazepine-epoxide and serotonin after systemic carbamazepine

Fig. 3 shows the brain extracellular concentrations of serotonin and of carbamazepine and its active metabolite carbamazepine-epoxide in dialysate from hippocampi of Sprague-Dawley rats. The left axis shows the uncorrected concentrations of carbamazepine and its metabolite while the right axis shows the concentrations after correction for in vivo probe recovery. The maximum concentration of carbamazepine (approximately 11 μM) occurred between 1 and 2 h after the i.p. administration of carbamazepine. The plateau in extracellular serotonin began between 2 and 3 h after the carbamazepine dose. This plateau in serotonin concentration occurs at the time of the maximal anticonvulsant effect of carbamazepine (Yan et al., 1992). Over the 12 h time period in which dialysis samples were obtained, both the concentration of carbamazepine and the concentration of serotonin returned to near-baseline levels. Basal serotonin release values in this experiment were 6.34 ± 0.39 fmol/sample.

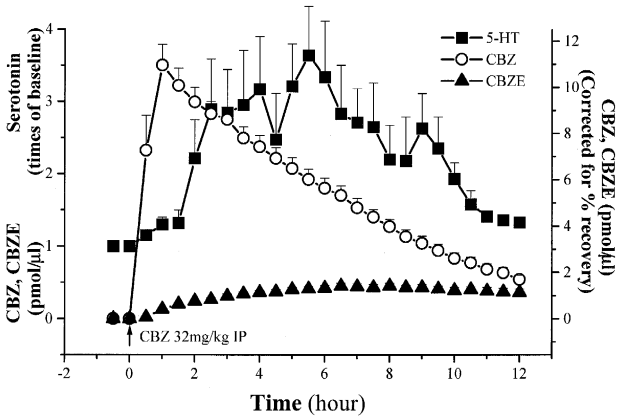

Fig. 3. Extracellular fluid concentrations (mean ± S.E.M.) of serotonin, carbamazepine (CBZ) and carbamazepine-epoxide (CBZE) in dialysate from hippocampi of Sprague-Dawley rats. The left axis shows the uncorrected concentrations of carbamazepine and carbamazepine-epoxide while the right axis shows the concentrations after correction for in vivo probe recovery. In vivo probe recovery averaged 32% for the probes used. Data depicted are from 8 animals.

3.5. Effect of focal carbamazepine administration on hippocampal extracellular serotonin

In order to assess its effect directly on serotonergic neuronal terminals, carbamazepine (as the inclusion complex in Molecusol) was perfused in increasing concentrations through the dialysis probe into the hippocampus. After three stable baseline samples (7.21 ± 0.21 fmol/sample) were obtained, the dialysate was switched from one containing only artificial CSF to one to which 50 μM carbamazepine had been added. After this response had stabilized, the dialysate was switched to one containing 250 μM carbamazepine and then subsequently to one containing 1250 μM carbamazepine. The data in Fig. 4a show that, when administered in this way, carbamazepine causes a concentration-related increase in extracellular serotonin. Each increase in carbamazepine concentration caused a corresponding increase in extracellular serotonin from the hippocampi of these animals. The concentrations delivered to the tissue after correction for probe recovery (16, 64 and 400 μM) are shown parenthetically in the figure. Fig. 4b shows that the area under the concentration–time curve increased with increasing carbamazepine concentration.

3.6. Effect of tetrodotoxin on focal carbamazepine-induced extracellular serotonin

Fig. 5 shows that focal administration of tetrodotoxin (1.5 μM) through the dialysis probe does not significantly alter the increase in hippocampal extracellular serotonin caused by focal administration of carbamazepine (250 μM) through the dialysis probe. In this experiment, carbamazepine was added to the artificial CSF after three stable baseline samples (10.49 ± 1.42 fmol/sample) had been

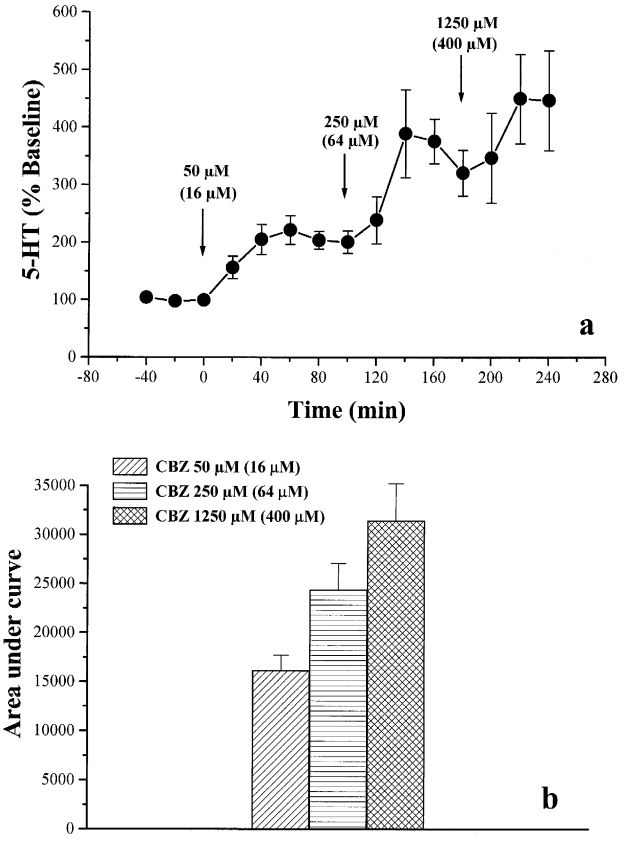

Fig. 4. (a) The effect on extracellular serotonin (mean ± S.E.M.) of carbamazepine (CBZ, complexed with Molecusol) administered through the dialysis probe placed in the hippocampus of Sprague-Dawley rats. The concentrations of CBZ in the dialysate (50, 250, 1250 μM) are shown in the figure as are the concentrations estimated to have been delivered to the tissue (16, 64, 400 μM). Estimations of concentrations delivered to the tissue are based on an in vivo probe recovery (loss) of 32.07 ± 1.26%. Carbamazepine produced a statistically significant treatment effect, $P < 0.001$ (ANOVA). (b) The area under the concentration–time curve for increases in extracellular serotonin following each of the CBZ concentrations. Data depicted are for 8 rats. $P = 0.0025$ (Pearson product moment) for the regression of dose vs. area under the concentration–time curve in panel b.

obtained. This concentration of carbamazepine had been shown to produce a significant but not maximal increase in extracellular serotonin (Fig. 4a,b). After the serotonin response had stabilized, the dialysate was switched to artificial CSF containing 250 μM carbamazepine and 1.5 μM tetrodotoxin. Addition of tetrodotoxin to the dialysate did not decrease the extracellular serotonin concentration. Finally, when the dialysate was switched back to artificial CSF, the hippocampal extracellular serotonin concentration returned to baseline.

3.7. Effect of zero calcium on focal carbamazepine-induced extracellular serotonin

Fig. 6 shows that elimination of calcium from the artificial CSF in the dialysate does not significantly alter the increase in hippocampal extracellular serotonin caused

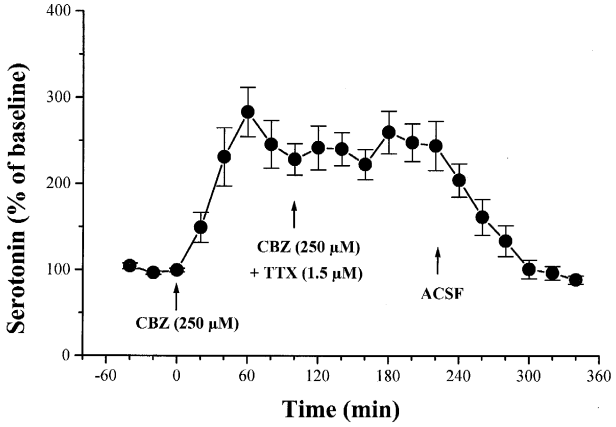

Fig. 5. Effect of focal infusion of carbamazepine (CBZ) or CBZ + 1.5 μM tetrodotoxin (TTX) though the dialysis probe placed in the hippocampus on extracellular serotonin (mean ± S.E.M.) in Sprague-Dawley rats. CBZ (250 μM) infusion began at time 0 and was continued for 90 min. At 90 min, the infusion solution was switched to 250 μM CBZ + 1.5 μM TTX. At 215 min, the perfusion solution was switched to artificial cerebrospinal fluid (ACSF). Data depicted are for 8 rats. Carbamazepine produced a statistically significant treatment effect, $P < 0.001$ (ANOVA).

by focal administration of carbamazepine (250 μM) through the dialysis probe. In this experiment, 250 μM carbamazepine produced approximately a 2.5-fold increase in hippocampal extracellular serotonin. Basal serotonin release was 12.87 ± 2.11 fmol/sample prior to the addition of carbamazepine to the dialysate. When the dialysate was switched to one containing 250 μM carbamazepine in artificial CSF from which calcium had been eliminated, there was no change in the extracellular serotonin concentration over the 100 min for which this solution was perfused. In order to ensure that tissue calcium concentra-

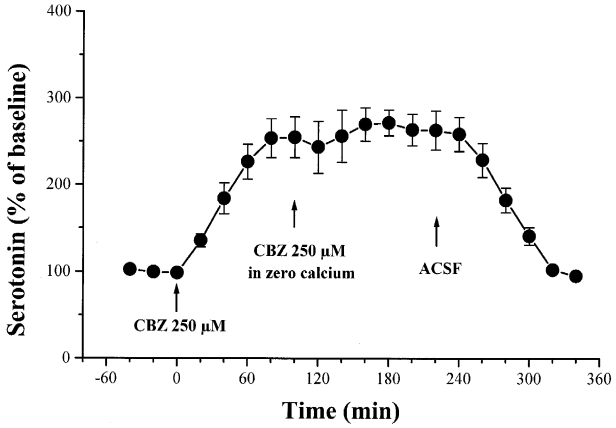

Fig. 6. Effect of focal infusion of carbamazepine (CBZ) through the dialysis probe placed in the hippocampus on extracellular serotonin (mean ± S.E.M.) in Sprague-Dawley rats. CBZ (250 μM) infusion began at time 0 and was continued for 90 min. At 90 min, the infusion solution was switched to 250 μM CBZ in a zero calcium artificial cerebrospinal fluid (ACSF) which also contained EGTA. At 215 min, the perfusion solution was switched to ACSF. Data depicted are for 8 rats. Carbamazepine produced a statistically significant treatment effect, $P < 0.001$ (ANOVA).

tion was substantially reduced in the perfused tissue surrounding the dialysis probe, EGTA (0.4 mM) was added to the zero calcium artificial CSF to chelate any remaining calcium. After the zero-calcium artificial CSF was perfused through the tissue for 100 min, the dialysate was switched to ordinary artificial CSF and the extracellular serotonin concentration returned to pre-treatment values.

4. Discussion

The present results demonstrate that systemic administration of carbamazepine causes a dose-related increase in extracellular serotonin in outbred Sprague-Dawley rats. If, as is the case in GEPRs, the increase in extracellular serotonin caused by carbamazepine is related to the anticonvulsant effect produced by this drug (Yan et al., 1992), then the effect of carbamazepine on extracellular serotonin in Sprague-Dawley rats should be dose related. In order to assure that we used doses of carbamazepine which are anticonvulsant in Sprague-Dawley rats, we determined the anticonvulsant ED_{50} for carbamazepine against maximal electroshock seizures. The ED_{50} dose for carbamazepine in this model is reported to be 7–10 mg/kg (Faigle and Feldman, 1989). In our hands, the anticonvulsant ED_{50} was determined to be 7.5 mg/kg. The doses used to evaluate the effect of systemic carbamazepine on extracellular serotonin (8, 16 and 32 mg/kg) were approximately the ED_{50} and two and four times the ED_{50} dose (Fig. 1). These results confirm our earlier findings in GEPRs (Yan et al., 1992) and suggest that the effect of anticonvulsant doses of carbamazepine to increase extracellular serotonin is a general pharmacodynamic property of this drug.

We chose the hippocampus as the location for our dialysis experiments for both pragmatic and theoretical reasons. The neuroanatomy of brain serotonergic system is such that nearly all of the cell bodies are located in the brainstem dorsal and median raphe nuclei. From these cell bodies, axons project forward and upward so that virtually the entire brain is innervated by serotonergic terminals projecting from the brainstem (Frazer and Hensler, 1994). Brain serotonergic innervation is uniform in that the concentration of serotonin across the brain and the density of serotonergic terminals is fairly consistent (Dailey et al., 1992a; Statnick et al., 1991). For our dialysis experiments, we wanted to sample a brain area that contains only serotonergic terminals and no serotonergic cell bodies. The hippocampus is far enough rostral that solute dialyzed out of the brain would be derived from nerve terminals and not be derived from serotonergic cell bodies in the raphe. We also wanted to sample from an area which is easily targeted by stereotaxic surgery and is broadly representative of the brain serotonergic terminal innervation. The hippocampus met these pragmatic and theoretical criteria.

Release of transmitters by brain neurons is closely connected with many other steps in neurotransmission. Included among these are transmitter synthesis, compartmentalization, reuptake and metabolism. Also, the release of one transmitter can be regulated by other neurotransmitters or neuromodulators. Many lines of evidence suggest that serotonin, like other biogenic amine neurotransmitters, is largely stored in synaptic vesicles within neuronal terminals. Thus, serotonin which is taken up by the active plasma membrane transporter and serotonin which is synthesized within the neuronal terminal is largely protected from intraneuronal monoamine oxidase. Intraneuronal serotonin which is also intravesicular can be released as unmetabolized transmitter by depolarizing stimuli via the process of exocytosis. Biogenic amine neurotransmitters can also be released by non-exocytotic processes. Release of cytoplasmic transmitter is thought to be mediated by the neuronal membrane transporter which exports the highly charged amine across the cell membrane (Raiteri et al., 1984). This process is often referred to as transporter reversal. Drugs such as fenfluramine cause serotonin release via transporter reversal. A third known mechanism by which drugs can increase extracellular serotonin is via blockade of the membrane transporter during ongoing neuronal impulse flow. Drugs such as the uptake blocker, fluoxetine, increase extracellular serotonin via this mechanism. Finally, a number of drugs increase extracellular serotonin via interactions with the transporter located in the membrane of synaptic vesicles. The classic monoamine depletor, reserpine, binds irreversibly to the vesicular transporter resulting in serotonin release (Schuldiner et al., 1993). Also, amphetamines and high concentrations of fenfluramine interact with the vesicular transporter to increase extracellular serotonin (Schuldiner et al., 1993; Rudnick and Wall, 1993).

In order to begin to determine the pharmacodynamic mechanism by which carbamazepine increases extracellular serotonin, we administered anticonvulsant doses of carbamazepine by i.p. injection and delivered tetrodotoxin through the dialysis probe placed in the hippocampus. In many respects, administration of drugs to a specific brain area through a dialysis probe is analogous to superfusion of a drug over a brain slice (Lindefors et al., 1989). In both procedures, the drug effect (in this case tetrodotoxin) is confined to a limited region of brain so that one can determine what effect the drug has on that particular brain structure. For a perfused brain area, the dialysis probe delivers drug to and removes solute from a cylindrical region surrounding the dialysis probe. For dialysis probes such as those used in our experiments 95% of the solute is delivered to and derived from a cylinder as high as the active dialysis area of the probe (3 mm) and 2–3 mm in diameter (Lindefors et al., 1989). The principal differences between a dialyzed area and a tissue slice are that the dialyzed region of brain remains connected to its blood supply. Also, primary neuronal connections are still present so that hormonal, neuromodulatory and local and distant neuronal influences remain intact.

When tetrodotoxin was administered through the dialysis probe, basal serotonin release was decreased significantly in a concentration-related fashion (compare in Fig. 2a,b). In contradistinction to the effect of tetrodotoxin on basal serotonin release, this neurotoxin did not decrease the release induced by systemic carbamazepine administration.

Tetrodotoxin blocks sodium channels in neuronal tissue. Exocytotic neurotransmitter release produced by nerve stimulation and nerve action potentials is diminished by tetrodotoxin administration through the dialysis probe (Chen and Reith, 1994; Sharp et al., 1990, 1989; Yan et al., 1994). In the present experiments, tetrodotoxin greatly reduced the basal release of serotonin, which is presumed to be dependent on action potentials. However, tetrodotoxin did not diminish the carbamazepine-induced increase in extracellular serotonin suggesting that this serotonin release is not dependent on nerve action potentials.

The possibility that carbamazepine acts on remote structures to induce serotonin release should be considered. For example, systemically administered carbamazepine could activate serotonergic neurons by an action on the cell bodies of these neurons in the raphe nuclei. Alternatively, carbamazepine could produce serotonin release indirectly by acting on non-serotonergic neurons which, in turn, activate serotonergic nerve terminals to cause serotonin release. The present data suggest that neither of these pharmacodynamic actions is entirely responsible for the carbamazepine-induced serotonin release. Perfusion of tetrodotoxin through the dialysis probe should virtually eliminate action potentials in the cylinder of tissue from which the released serotonin is being dialyzed. Thus, action potentials travelling along the serotonergic neurons arising in the raphe nuclei should stop before reaching the serotonergic terminals in the 3 mm diameter cylinder of tissue being perfused. Also, the tetrodotoxin perfusion should effectively prevent action potentials in non-serotonergic neurons within the perfused cylinder. Thus, these data suggest that a major part of the effect of carbamazepine to release serotonin is by an action directly on the serotonergic terminals in the perfused cylinder of tissue. Because action potentials are a normal component of exocytotic neurotransmitter release, these data also suggest that the carbamazepine-induced increase in extracellular serotonin is not mediated by exocytosis.

In order to verify further that the action of carbamazepine to increase extracellular serotonin is mediated by an effect directly on serotonergic terminals, we administered carbamazepine through a dialysis probe placed in the hippocampus. When drugs are administered in this way, drug concentrations delivered to the tissue are highest in the area closest to the dialysis membrane and diminish as the distance from the probe increases. Pharmacodynamically active concentrations of the drug are delivered to the same volume of tissue from which serotonin and other solutes are dialyzed out. Because the total amount of drug delivered to the animal is very small, drug concentrations in tissues other than those in close proximity to the probe are negligible. The data shown in Fig. 4a,b confirm that carbamazepine can increase extracellular serotonin via an action directly on the serotonergic terminals present in the hippocampus. Further, we were able to show that the effect of focally administered carbamazepine on extracellular serotonin is not diminished by tetrodotoxin or by removing calcium from the dialysate (Figs. 5 and 6).

It is possible that systemically administered and focally administered carbamazepine do not increase extracellular serotonin by identical cellular mechanisms. This possibility is supported by the fact that the brain extracellular concentrations achieved after systemic and focal administration of carbamazepine are different. After the largest systemic carbamazepine dose administered in this study (32 mg/kg i.p.), the maximum extracellular hippocampal carbamazepine concentration was approximately 11 μM (Fig. 3). This concentration is within the range of carbamazepine concentrations found in human CSF (roughly 2–20 μM) during treatment with therapeutic doses of carbamazepine (Rogawski and Porter, 1990; Rall and Schleifer, 1990). Since the dialysis probes used in the present study had an efficiency of approximately 32% at delivering carbamazepine to the tissue, the concentrations delivered to the tissue by focal carbamazepine administration (Fig. 5) would have been approximately 16, 64 and 400 μM. Thus, it seems possible that systemic carbamazepine administration may produce cellular effects to release serotonin which are not produced when the drug is administered focally to the terminal fields. Perhaps an effect of carbamazepine on the cell body or axon of the serotonergic neurons is additive with the effect of carbamazepine on the serotonergic terminals in the hippocampus. This may account for the fact that systemic carbamazepine releases serotonin at extracellular carbamazepine concentrations which are much less effective when they are given focally. Interestingly, we have found that concentrations of carbamazepine required to release serotonin when it is given focally are similar to the concentrations required to release serotonin from tissue slices in vitro (Dailey et al., 1997).

One further feature of the data depicted in Fig. 3 is worthy of note. After i.p. administration of carbamazepine, the maximal increase in extracellular carbamazepine concentration in the hippocampus occurred between 1 and 2 h after the drug was administered. The extracellular serotonin concentration in these same samples reached and maintained a plateau which was delayed when compared with the carbamazepine concentration. The plateau for extracellular serotonin was reached approximately 2–3 h after the administration of carbamazepine. Interestingly, the maximal anticonvulsant effect of carbamazepine and the maximal increase in extracellular serotonin occur 2–3 h after i.p. carbamazepine in GEPRs (Yan et al., 1992).

Although carbamazepine at high submillimolar concen-

trations has been reported to block sodium channels as do local anesthetics (Smith et al., 1977), this activity is probably not involved at the doses administered in the present experiments.

Three other processes by which carbamazepine possibly could increase extracellular serotonin are: (1) blockade of serotonin reuptake at the cell membrane (membrane transporter blockade), (2) interaction with the vesicular transporter, and (3) membrane transporter reversal. It seems unlikely that carbamazepine increases extracellular serotonin by blocking reuptake because blockade of reuptake prevents re-entry of serotonin that has been released physiologically by exocytosis. As can be seen from the data presented in Fig. 2, tetrodotoxin greatly reduced basal serotonin release which is caused by exocytosis. If carbamazepine were blocking reuptake, one would expect tetrodotoxin to decrease the carbamazepine-induced increase in extracellular serotonin because of tetrodotoxin's effect to decrease exocytotic release of serotonin. Also, we have shown previously that the increase in extracellular serotonin caused by local perfusion of cocaine (Chen and Reith, 1994) and by systemic administration of a combination of fluoxetine and the serotonin precursor, 5-hydroxytryptophan, is greatly decreased by tetrodotoxin (Yan et al., 1994).

The present results do not rule out a possible action of carbamazepine involving release directly from storage vesicles. Similarly, these results do not eliminate the possibility that carbamazepine releases serotonin via reversal of the plasma membrane transporter.

Acknowledgements

Supported in part by NS32628. The authors are indebted to Annette Burk for her very valuable analytical support and to Kevin R. Steidley for his excellent assistance in preparing the manuscript.

References

Bourgeois, B.F., Wad, N., 1984. Individual and combined antiepileptic and neurotoxic activity of carbamazepine and carbamazepine-10,11-epoxide in mice. J. Pharmacol. Exp. Ther. 231, 411–415.

Browning, R.A., 1987. The role of neurotransmitters in electroshock seizure models. In: Jobe, P.C., Laird, H.E. (Eds.), Neurotransmitters and Epilepsy. Humana Press, Clifton, NJ, pp. 277–320.

Browning, R.A., Hoffmann, W.E., Simonton, R.L., 1978. Changes in seizure susceptibility after intracerebral treatment with 5,7-dihydroxytryptamine: role of serotonergic neurons. Ann. NY Acad. Sci. 305, 437–456.

Buterbaugh, G.G., 1977. A role for central serotonergic systems in the pattern and intensity of the convulsive response of rats to electroshock. Neuropharmacology 16, 707–709.

Chen, N.H., Reith, M.E.A., 1994. Effects of locally applied cocaine, lidocaine, and various uptake blockers on monoamine transmission in the ventral tegmental area of freely moving rats: a microdialysis study on monoamine interrelationships. J. Neurochem. 63, 1701–1713.

Dailey, J.W., Reigel, C.E., Mishra, P.K., Jobe, P.C., 1989. Neurobiology of seizure predisposition in the genetically epilepsy-prone rat. Epilepsy Res. 3, 3–17.

Dailey, J.W., Mishra, P.K., Ko, K.H., Penny, J.E., Jobe, P.C., 1991. Noradrenergic abnormalities in the central nervous system of seizure-naive genetically epilepsy-prone rats. Epilepsia 32, 168–173.

Dailey, J.W., Mishra, P.K., Ko, K.H., Penny, J.E., Jobe, P.C., 1992a. Serotonergic abnormalities in the central nervous system of seizure-naive genetically epilepsy-prone rats. Life Sci. 50, 319–326.

Dailey, J.W., Yan, Q.S., Mishra, P.K., Burger, R.L., Jobe, P.C., 1992b. Effects of fluoxetine on convulsions and on brain serotonin as detected by microdialysis in genetically epilepsy-prone rats. J. Pharmacol. Exp. Ther. 260, 533–540.

Dailey, J.W., Steidley, K.R., Milbrandt, J., Reith, M.E.A., Jobe, P.C., 1997. Carbamazepine induced serotonin (5-HT) release from hippocampal slices: mechanistics studies. Pharmacologist 39, 346, Abstract.

Faigle, J.W., Feldman, K.F., 1989. Carbamazepine biotransformation. In: Levy, R. et al. (Eds.), Antiepileptic Drugs. Raven Press, New York, NY, Vol. 3, pp. 491–504.

Frazer, A., Hensler, J.G., 1994. Serotonin. In: Siegel, G.J. et al. (Eds.), Basic Neurochemistry. Raven Press, New York, NY, Vol. 5, pp. 283–308.

Hiramatsu, M., Ogawa, K., Kabuto, H., Mori, A., 1987. Reduced uptake and release of 5-hydroxytryptamine and taurine in the cerebral cortex of epileptic El mice. Epilepsy Res. 1, 40–45.

Jobe, P.C., Picchioni, A.L., Chin, L., 1973a. Role of brain norepinephrine in audiogenic seizure in the rat. J. Pharmacol. Exp. Ther. 184, 1–10.

Jobe, P.C., Picchioni, A.L., Chin, L., 1973b. Role of brain 5-hydroxytryptamine in audiogenic seizure in the rat. Life Sci. 13, 1–13.

Jobe, P.C., Laird, H.E., Ko, K.H., Ray, T., Dailey, J.W., 1982. Abnormalities in monoamine levels in the central nervous system of the genetically epilepsy-prone rat. Epilepsia 23, 359–366.

Jobe, P.C., Dailey, J.W., Reigel, C.E., 1986. Noradrenergic and serotonergic determinants of seizure susceptibility and severity in genetically epilepsy-prone rats. Life Sci. 39, 775–782.

Jobe, P.C., Lasley, S.M., Burger, R.L., Bettendorf, A.F., Mishra, P.K., Dailey, J.W., 1992. Absence of an effect of aspartame on seizures induced by electroshock in epileptic and non-epileptic rats. Amino Acids 3, 155–172.

Laird, H.E., Dailey, J.W., Jobe, P.C., 1984. Neurotransmitter abnormalities in genetically epileptic rodents. Fed. Proc. 43, 2505–2509.

Le Quellec, A., Dupin, S., Genissel, P., Saivin, S., Marchand, B., Houin, G., 1995. Microdialysis probes calibration – gradient and tissue dependent changes in no net flux and reverse dialysis methods. J. Pharmacol. Toxicol. Methods 33, 11–16.

Lindefors, N., Amberg, G., Ungerstedt, U., 1989. Intracerebral microdialysisI. Experimental studies of diffusion kinetics. J. Pharmacol. Methods 22, 141–156.

Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Pasini, A., Tortorella, A., Gale, K., 1992. Anticonvulsant effect of intranigral fluoxetine. Brain Res. 593, 287–290.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press Australia, Sydney, p. vii.

Prendiville, S., Gale, K., 1993. Anticonvulsant effect of fluoxetine on focally evoked limbic motor seizures in rats. Epilepsia 34, 381–384.

Raiteri, M., Marchi, M., Maura, G., 1984. Release of catecholamines, serotonin, and acetylcholine from isolated brain tissue. In: Lajtha, A. (Ed.), Handbook of Neurochemistry. Plenum, New York, NY, pp. 431–462.

Rall, T.W., Schleifer, L.S., 1990. Drugs effective in the therapy of the epilepsies. In: Gilman, A.G. et al. (Eds.), The Pharmacological Basis of Therapeutics, Pergamon Press, New York, NY, pp. 436–462.

Reigel, C.E., Dailey, J.W., Jobe, P.C., 1986. The genetically epilepsy-prone rat: an overview of seizure-prone characteristics and responsiveness to anticonvulsant drugs. Life Sci. 39, 763–774.

Rogawski, M.A., Porter, R.J., 1990. Antiepileptic drugs: pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. Pharmacol. Rev. 42, 223–286.

Rudnick, G., Wall, S.C., 1993. Non-neurotoxic amphetamine derivatives release serotonin through serotonin transporters. Mol. Pharmacol. 43, 271–276.

Scheyer, R.D., During, M.J., Cramer, J.A., Toftness, B.R., Hochholzer, J.M., Mattson, R.H., 1994. Simultaneous hplc analysis of carbamazepine and carbamazepine epoxide in human brain microdialysate. J. Liq. Chromatogr. 17, 1567–1576.

Schuldiner, S., Steiner-Mordoch, S., Yelin, R., Wall, S.C., Rudnick, G., 1993. Amphetamine derivatives interact with both plasma membrane and secretory vesicle biogenic amine transporters. Mol. Pharmacol. 44, 1227–1231.

Sharp, T., Bramwell, S.R., Clark, D., Grahame Smith, D.G., 1989. In vivo measurement of extracellular 5-hydroxytryptamine in hippocampus of the anaesthetized rat using microdialysis: changes in relation to 5-hydroxytryptaminergic neuronal activity. J. Neurochem. 53, 234–240.

Sharp, T., Bramwell, S.R., Grahame Smith, D.G., 1990. Release of endogenous 5-hydroxytryptamine in rat ventral hippocampus evoked by electrical stimulation of the dorsal raphe nucleus as detected by microdialysis: sensitivity to tetrodotoxin, calcium and calcium antagonists. Neuroscience 39, 629–637.

Smith, J.E., Lane, J.D., Shea, P.A., McBride, W.J., 1977. Neurochemical changes following the administration of precursors of biogenic amines. Life Sci. 21, 301–306.

Sparks, D.L., Buckholtz, N.S., 1985. Combined inhibition of serotonin uptake and oxidative deamination attenuates audiogenic seizures in DBA/2J mice. Pharmacol. Biochem. Behav. 23, 753–757.

Statnick, M.A., Dailey, J.W., Jobe, P.C., Browning, R.A., 1991. Abnormalities in brain serotonin uptake and steady state concentration in the genetically epilepsy-prone rat (GEPR). Soc. Neurosci. Abstr. 17, 171, Abstract.

Swinyard, E.A., 1972. Electrically induced convulsions. In: Purpura, D.P. et al. (Eds.), Experimental Models of Epilepsy – A Manual for Laboratory Worker. Raven Press, New York, NY, pp. 433–458.

Wada, Y., Hasegawa, H., Nakamura, M., Yamaguchi, N., 1992a. Serotonergic inhibition of limbic and thalamic seizures in cats. Neuropsychobiology 25, 87–90.

Wada, Y., Nakamura, M., Hasegawa, H., Yamaguchi, N., 1992b. Role of serotonin receptor subtype in seizures kindled from the feline hippocampus. Neurosci. Lett. 141, 21–24.

Yan, Q.S., Mishra, P.K., Burger, R.L., Bettendorf, A.F., Jobe, P.C., Dailey, J.W., 1992. Evidence that carbamazepine and antiepilepsirine may produce a component of their anticonvulsant effects by activating serotonergic neurons in genetically epilepsy-prone rats. J. Pharmacol. Exp. Ther. 261, 652–659.

Yan, Q.S., Jobe, P.C., Dailey, J.W., 1994. Evidence that a serotonergic mechanism is involved in the anticonvulsant effect of fluoxetine in genetically epilepsy-prone rats. Eur. J. Pharmacol. 252, 105–112.