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STUDIES ON THE INTRACEREBRAL METABOLISM OF ANTICONVULSANT DRUGS—II. DISPOSITION OF CARBAMAZEPINE IN THE ISOLATED PERFUSED RAT BRAIN

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Abstract—Carbamazepine (CBZ) was perfused (85 nmoles/ml) through the isolated brains of rats. After 2 hr the mean regional concentrations of the drug were between 170 and 234 nmoles/g wet weight. The total brain content of CBZ was 390 nmoles. During perfusion 82 nmoles epoxycarbamazepine (E-CBZ) were formed, most of which were found in perfusion medium. Tissue levels of E-CBZ were between 0.3 and 2.8 nmoles/g wet weight. No dihydroxycarbamazepine (DH-CBZ) could be found. Pretreatment of the rats with phenobarbital neither influenced the uptake of CBZ into the brains nor increased the formation of E-CBZ significantly.

In man, as in animals, carbamazepine is almost completely metabolized. It is suggested that the epoxidation of the molecule to 10,11-epoxycarbamazepine (E-CBZ) with subsequent hydratation to trans-10,11-dihydro-10,11-dihydroxycarbamazepine (DH-CBZ) is the major pathway [1]. The reaction leading to E-CBZ is catalyzed by hepatic monooxygenases [2]. From E-CBZ the trans-diol metabolite is formed enzymatically by liver epoxide hydratase [3].

After single oral doses of CBZ to healthy subjects, the average half-life of elimination from plasma was found to be 37.5 hr [4]. However, daily administration of CBZ over 2 to 3 weeks reduced the half-life by one third; with long-term monotherapy with CBZ it was reduced by about a half. In patients receiving concomitant treatment with certain other anticonvulsants (e.g. phenytoin, phenobarbital, primidone), elimination of CBZ was further accelerated (for reference see [1]). Additionally, the ratio of E-CBZ to CBZ in the plasma of patients taking other anticonvulsants concomitantly was significantly higher as compared to patients receiving solely CBZ [5]; the equipotent anticonvulsant properties of E-CBZ as compared to the parent drug has been noted in various animal models [6]. In addition, the efficacy of E-CBZ in trigeminal neuralgia has been demonstrated [7].

Under steady state conditions in man, almost 60% of the daily dose of carbamazepine (CBZ) is excreted in urine as products of the epoxide pathway [8]. In epileptic patients undergoing chronic treatment, about 32–61% of the daily dose is excreted as DH-CBZ, 1–1.5% as E-CBZ, and 0.5% as unmetabolized CBZ [8].

It was shown, that pretreatment of rats with CBZ resulted in an increased oxidation of CBZ *in vitro*. Under similar conditions epoxide hydratase could also be induced (for reference see [3]). Perfusion studies with livers from rats pretreated with phenobarbital [9] indicated an accelerated metabolism of CBZ compared to perfusions of livers from control rats. The enzymes involved in the epoxide pathway of CBZ are not only induced by CBZ, but also by E-CBZ itself, the induction of monooxygenase being greater than that of epoxide hydratase or glutathione *S*-transferase [3].

The metabolic transformation of primidone to the anticonvulsant drugs phenobarbital and phenylethylmalonic acid in the isolated brain of the rat has recently been noted [10]. It was speculated that the intracerebral formation of an anticonvulsive metabolite, which means a formation at the site of action, may influence the antiepileptic effect of the metabolite.

In the present study, carbamazepine was perfused through the isolated rat brain in order to investigate the intracerebral metabolism of the drug. To gather information about induction of metabolizing enzymes in the brain, brains of untreated animals and those of animals pretreated with phenobarbital were perfused.

MATERIALS AND METHODS

Chemicals. All solvents and reagents used were of p.a.-grade or of HPLC-purity (Merck, Darmstadt, and C. Roth, Karlsruhe, F.R.G.). Carbamazepine (CBZ, 5-carbamoyl-5H-dibenzo[b,f]azepine), 10,11-epoxycarbamazepine (E-CBZ) and 10,11-dihydro-10,11-dihydroxycarbamazepine (DH-CBZ) were kindly donated by Ciba-Geigy (Basel, Switzerland).

5-(*p*-methylphenyl)-5-phenylhydantoin and phenobarbital were purchased from Serva (Heidelberg, F.R.G.). Fluorocarbon FC 43 was from 3M

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Company (Neuss, F.R.G.); and Pluronic F 68 from Erbslöh (Düsseldorf, F.R.G.).

Perfusion technique. Male Sprague Dawley rats (200–210 g; S. Ivanovas, Kisslegg, F.R.G.) were anaesthetized by intraperitoneal injection of urethane (0.9 g/kg body wt) and atropine (0.01 mg/kg). The operation procedure was performed as described earlier [10, 11]. Perfusion fluid entered the brain through the internal carotid arteries which were cannulated with PE 50 tubes. After commencement of perfusion, the neck was ligated to prevent leakage of perfusion fluid through the vertebral arteries. The perfusion medium left the brain through the jugular veins.

As perfusion medium a fluorocarbon emulsion with pluronic F 68 as macromolecular component was taken. The water phase of the emulsion contained the salts of Krebs bicarbonate buffer (medium I) [12] or, instead of bicarbonate, Tris buffer (medium II). The composition of medium II was as follows: instead of 27.8 mmoles/l $NaHCO_3$ 27.8 mmoles/l Tris-(hydroxymethyl)-aminomethan was added and NaH_2PO_4 was taken in place of KH_2PO_4. For details see [13]. During perfusion, medium I was gassed with carbogen (95% O_2, 5% CO_2) and medium II with oxygen.

Perfusions were carried out at 28° as cyclic perfusion (2 hr) using pulsatory perfusion pressure (systolic pressure: 120 mmHg, diastolic pressure: 100 mmHg, pulse rate: 280/min).

The functioning state of the brains were monitored measuring the following parameters: EEG, flow rate, mean perfusion pressure and oxygen consumption. The molar ratios of ATP to ADP and lactate to pyruvate in the brain tissue were determined. For further details see [11].

Carbamazepine, 13.6 μmoles, was dissolved in 160 ml perfusion medium using ultrasonication. The concentration of the drug in the perfusion medium was 85 nmoles/ml.

After perfusion, the brains were quickly removed from the skulls, cooled to 4° and prepared into 10 regions: cerebral cortex, cerebellum, corpus striatum, septum, thalamus, hippocampi, hypothalamus, hypophysis, mesencephalon and pons/medulla oblongata.

Parallel to this, control perfusions of CBZ without brains were carried out to assess the amount of E-CBZ formed non-enzymically during brain perfusion.

Extraction and purification of drugs. The different brain regions were weighed and separately homogenized in 3 ml methanol. A quantity of 1.5 ng internal standard [(5-(*p*-methylphenyl)-5-phenylhydantoin)] was added per mg tissue. The homogenate was centrifuged at 3000 g_{av} for 10 min. The pellet was washed with 0.5 ml methanol and again centrifuged at 3000 g_{av}. The methanol phases were combined, evaporated to dryness under vacuum and the residue dissolved in 5 ml water. The aqueous solution was extracted 3 times with the same volume of methylene chloride. The organic phases were combined and evaporated to dryness under vacuum. The residue was dissolved in acetonitrile/methanol/water (23.1 : 3.6 : 73.3, by vol.).

The perfusion medium was worked up in the following manner: 150 ng internal standard were given to 200 μl perfusion medium. After the addition of 4 ml methanol, the emulsion disaggregated and two phases were formed. The fluorocarbon phase was discarded and the methanol/water-phase was evaporated to dryness under vacuum. The residue was dissolved in 4 ml water which was subsequently extracted 3 times with the same volume of methylene chloride. The organic phases were combined, evaporated to dryness under vacuum and the residue dissolved in acetonitrile/methanol/water (23.1 : 3.6 : 73.3, by vol.).

High pressure liquid chromatography. The equipment was purchased from Spectra Physics, Darmstadt, F.R.G. The variable wavelength u.v.-detector was operated at 220 nm. Injections were achieved with a high pressure sample injection valve (Rheodyne 7125 c/o Latek, Heidelberg, F.R.G.), fitted with a 20 μl sample loop. Chromatographic run was performed at 50°.

Before use, the mobile phase was degassed by ultrasonication; during chromatography the mobile phase was continuously gassed with helium. Chromatograms were recorded and computed by a SP 4100 integrator.

Carbamazepine and its epoxide were separated from each other on a RP 18 column (Nucleosil, 7 μm, 150 × 4.6 mm, Macherey and Nagel, Düren, F.R.G.), using acetonitrile/methanol/water (23.1/3.6/73.3, by vol.) as mobile phase; 5-(*p*-methylphenyl)-5-phenylhydantoin was used as internal standard.

For definite identification the respective peaks of CBZ and E-CBZ were fractionated and submitted to mass spectrometry (LKB 9000, direct inlet, sample temperature 100°, ion source temperature 270°, electron energy 25 eV).

Equilibrium dialysis. Binding of carbamazepine in the perfusion medium was determined by equilibrium dialysis. The dialysis was carried out at 28° using a Dianorm BFD (Diachemie, Zürich, Switzerland), with a Visking membrane (Serva). For further details, see [14].

Application of phenobarbital to rats. Phenobarbital was applied for 14 days to the rats via their drinking water. The drug was dissolved in water (500 mg/l) and offered to the rats ad libitum. Perfusions were carried out 36–48 hr after having switched to pure drinking water.

To assess enzyme induction by phenobarbital, livers of treated and control animals were perfused with CBZ. The metabolism of CBZ was substantially higher in the treated group (results not given).

RESULTS

Functional parameters of the isolated brains. During perfusion, the EEG of the isolated brains exhibited a regular frequency of 14–16 Hz. Lower frequencies (2–4/sec) could be seen sporadically during the first 10 min of perfusion. During perfusion, the mean vascular resistance (calculated from the flow rate and the mean perfusion pressure) and the oxygen consumption remained constant, indicating that there was no edema. Additionally, the molar ratios of ATP to ADP as well as lactate to pyruvate

Table 1. Distribution of CBZ and E-CBZ in the isolated brain of the rat after perfusion of CBZ (85 nmoles/ml) for 2 hr.

Regions	Uptake of CBZ		Formation of E-CBZ	
	No treatment	Phenobarbital pretreatment	No treatment	Phenobarbital pretreatment
	(pmoles/mg)		(pmoles/mg)	
Cerebral cortex	228 ± 39	209 ± 44	0.6 ± 0.2	0.6 ± 0.2
Cerebellum	185 ± 30	231 ± 48	0.4 ± 0.2	0.4 ± 0.1
Corpus striatum	186 ± 21	209 ± 39	0.7 ± 0.3	0.5 ± 0.2
Septum	201 ± 54	189 ± 65	1.5 ± 0.3	0.9 ± 0.2
Thalamus	224 ± 56	225 ± 45	0.5 ± 0.2	0.5 ± 0.1
Hippocampi	170 ± 17	201 ± 42	0.3 ± 0.1	0.4 ± 0.1
Hypothalamus	195 ± 40	199 ± 27	1.8 ± 0.6	1.2 ± 0.3
Hypophysis	191 ± 31	220 ± 56	2.2 ± 0.8	2.8 ± 1.0
Mesencephalon	195 ± 4	240 ± 59	0.7 ± 0.2	0.4 ± 0.1
Pons/medulla oblongata	234 ± 33	230 ± 32	0.3 ± 0.1	0.4 ± 0.1
			(pmoles/μl)	
Perfusion medium			0.5 ± 0.1	0.5 ± 0.1

Perfusion studies were carried out with medium I. Mean values ± S.D. (N = 6) are given. For further details see text.

were close to values measured *in vivo* thus indicating no lack of high energy phosphates or acidosis. For details see [11].

Uptake of CBZ into the brain. After 2 hr of perfusion the concentrations of CBZ and its epoxide in the brains were determined. The values obtained in perfusion studies with medium I are indicated in Table 1.

In total about 384 nmoles of CBZ were taken up into the brains of untreated rats; the corresponding value of pretreated rats was 405 nmoles. The difference is statistically not significant. In comparison to other brain regions, the concentrations of CBZ in the cerebral cortex and thalamus were slightly increased. In each region the tissue concentration of CBZ was far above that in the perfusion medium (85 pmoles/μl) indicating preferential uptake of the agent into the brain. After perfusion with medium II, total uptake as well as regional distribution of CBZ in the brains was very similar compared to data obtained during perfusions with medium I (values not given).

After pretreatment of the rats with phenobarbital neither the total uptake of CBZ nor the regional distribution of the substance into the brain changed significantly (see Table 1).

Binding of CBZ in the perfusion medium. The binding of CBZ as well as E-CBZ in the perfusion medium was determined by equilibrium dialysis. It was found to be between 85 and 90% for both drugs (concentration range: 1–100 nmoles/ml).

Metabolism of CBZ in the brain. After perfusion about 82 nmoles E-CBZ were found in the brain tissue and the perfusion medium. In most of the brain regions the concentration of E-CBZ was similar to that in perfusion medium while in hypophysis, hypothalamus and septum considerably higher concentrations could be detected. The values are given in Table 1. After pretreatment with phenobarbital, similar amounts of E-CBZ were found, indicating that formation of the metabolite in the brain was not increased.

In all perfusion experiments the formation of DH-CBZ could not be detected.

In control perfusions without brains neither E-CBZ nor DH-CBZ were formed.

DISCUSSION

In the present study it could be noticed for the first time that CBZ is metabolized to its epoxide in the rat brain. This finding was obtained by using the isolated perfused brain preparation which offers the opportunity to investigate cerebral metabolism unaffected by extracranial organs.

The reaction leading to E-CBZ represents the major pathway of biotransformation of CBZ. After pretreatment with CBZ itself or other anticonvulsants, epoxidation increases in man, rat and other species [6, 8, 9, 15]. The anticonvulsant activity of E-CBZ was found to be comparable to that of the parent drug in various animal models, while the hydratation product DH-CBZ exhibited low anti-epileptic effects [6].

After single oral dosing in rats the brain to plasma ratio was found to be different for CBZ and its epoxide. The brain concentrations of CBZ were 1.1 to 1.6 times the plasma concentration. In contrast, the corresponding values for E-CBZ (0.4–0.8) suggested a reduced penetration of the metabolite into the brain (see [1]). However, after chronic treatment with carbamazepine in epileptic patients, a brain/plasma ratio of 1.1–1.2 could be observed for CBZ as well as for E-CBZ [16].

After acute application of CBZ to rats regional brain distribution was studied (see [1]). Higher concentrations were found in the cortex, thalamus and hippocampus, lower levels were observed in the cerebellum and lower brainstem. With time there was a leveling of regional drug concentrations, subcortical

structures having the highest relative drug concentrations.

Anticonvulsive threshold concentrations (maximal electroshock) were in the same order of 15 to 19 pmoles/mg for both carbamazepine and the epoxide (see [1]). However, after intraperitoneal b.i.d. application of 25 mg CBZ/kg to rats—a quantity which corresponds to the therapeutic dosage in man [17]—mean brain levels were about 140 pmoles/mg wet weight and mean plasma levels were about 110 pmoles/μl [15]. In the present study (85 pmoles/μl in the perfusion medium) higher tissue levels of 170 to 234 pmoles/mg were obtained. The binding of CBZ to the perfusion medium (about 90%) was in the same range compared to data obtained with plasma proteins [18] and cannot explain these differences.

After 2 hr of perfusion the highest concentrations of CBZ were found in the cerebral cortex, thalamus and pons/medulla oblongata. However, the CBZ-contents of the various brain regions did not differ significantly. The total uptake of CBZ was slightly but not significantly increased after pretreatment of the rats with phenobarbital, a drug which strongly induces the epoxide pathway in the liver [9, 19]. The highest concentrations of the epoxide were found in the hypophysis, hypothalamus and septum. These regions seem to possess the highest activities for drug metabolizing enzymes. In brain perfusion studies with oestradiol-17β [20] and primidone [10] the highest concentrations of the respective metabolites were found in the above mentioned regions.

In total, about 82 nmoles of E-CBZ were formed during perfusion, most of which (about 98%) were found in the perfusion medium. This strongly suggests that the epoxide was extracted from the brain during perfusion by the perfusion medium. A similar effect could be observed earlier in liver perfusion studies using a fluorocarbon medium [21].

The intracerebral turnover of CBZ is about 30 times higher compared to the turnover observed with primidone in an earlier brain perfusion study [10]. However, the tissue levels of E-CBZ reached were far below the anticonvulsant threshold of about 15 pmoles/mg for E-CBZ (see [1]). When evaluating these data it should be considered that the metabolite was almost completely extracted from the brain by the perfusion medium. In the intact organism, when E-CBZ is also formed by the liver, the local concentrations in the brain of E-CBZ, built up by intracerebral oxidation of CBZ, may be substantially higher.

In conclusion, from the results presented here, it may be speculated that the intracerebral oxidation of CBZ contributes to the intracerebral, anticonvulsant effective concentration of E-CBZ. However, considering the substantial amounts of E-CBZ formed by the liver during chronic treatment with CBZ, this conclusion is speculative and needs to be supported by further studies.

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