Effect of Chronic Hypertension on the Blood–Brain Barrier Permeability of Libenzapril

Jian-ping Tang,[1] Ashok Rakhit,[1,2] Frank L. Douglas,[2] and Srikumaran Melethil[1,3]

Received May 29, 1991; accepted September 2, 1991

Very little information is available on the permeability of the blood–brain barrier (BBB) to small polar drugs in chronic hypertension. The blood and cerebrospinal fluid (CSF) pharmacokinetics of libenzapril (LZP), a potent angiotensin converting enzyme inhibitor, were investigated in hypertensive (SH) and normotensive (SD) rats. Following intravenous bolus administration of this hydrophilic drug, the terminal rate constant for elimination (β), steady-state volume of distribution ($V_{d_{ss}}$), and systemic clearance (CL) were similar in these two animal groups. Other pharmacokinetic parameters (C_p^o, α, k_{12}, and k_{21}) were significantly ($P < 0.05$) greater in the hypertensive group, except for the volume of the central compartment (V_c) and ratio of V_c to $V_{d_{ss}}$, which were smaller in SH rats. The ratio of area under the concentration–time curve (AUC) in CSF to blood was about twofold higher in SH rats compared to normotensive rats, showing increased BBB permeability in hypertensive rats. An acute brain uptake study was also performed in SH, SD, and WK rats by intracarotid administration of ^{14}C-LZP along with ^3H_2O as a reference marker. Both LZP and water transport was found to be significantly higher (about two- to five-fold) in six of the seven different brain regions in SH rats as compared to the normotensive (SD and WK) controls. Because of this simultaneous increase in concentrations of both the drug and the reference marker, BUI values were not affected. Regional brain concentrations in SH rats were also linearly correlated with the mean arterial pressure (MAP) values, providing further evidence of the systemic pressure related increase in BBB permeability.

KEY WORDS: blood–brain barrier (BBB); pharmacokinetics; libenzapril; cerebrospinal fluid (CSF); chronic hypertension.

INTRODUCTION

One basic function of the blood–brain barrier (BBB) is to protect the brain and cerebrospinal fluid (CSF) compartments of the central nervous system from the systemic (blood) environment by restricting entry of solutes of polar nonelectrolytes through intercellular tight junctions at the cerebral capillaries. However, there is strong evidence that this barrier becomes more permeable to large molecules (molecular weight, 4000–70,000) such as albumin and dextran under conditions of acute hypertension (1–4). In contrast, relatively few reports are available on the evaluation of BBB permeability in chronic hypertension (5,6). Mueller and Heistad (5) found no differences in brain permeability between normotensive (WK) and SH rats to albumin. Majack and Bhalla (6) examined both paracellular and pinocytotic transport of horseradish peroxidase across the middle cerebral artery in SH and normotensive rats. Interendothelial junctions in both groups demonstrated impermeability to the tracer, which led them to conclude that, unlike acute hypertension, animals with chronic hypertension may not be characterized by increased BBB permeability.

No information is known about the effect of chronic hypertension on the BBB permeability of commonly used drugs, which are mostly small molecules (molecular weight <1000). Since hypertension affects about 15 to 20% of Americans (7), the present investigation is of both scientific interest and potential clinical significance. The major objectives of this study were to compare (i) the blood and CSF pharmacokinetic behavior of libenzapril (LZP), a potent angiotensin converting enzyme inhibitor, in normotensive (SD) and the chronic hypertensive (SH) rats and (ii) the brain uptake of LZP between normotensive (SD and WK) and chronic hypertensive (SH) rats. The blood–brain permeability of LZP evaluated in this commonly used chronic hypertensive (SH rat) model (4,6,8,9) was significantly greater compared to normotensive (SD and WK rats) controls.

MATERIALS AND METHODS

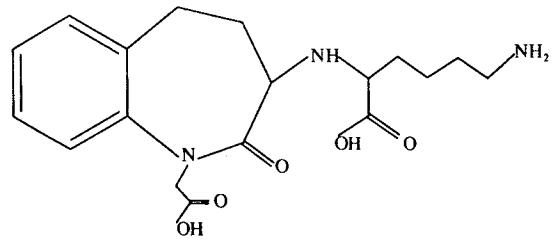
Radioactive Materials. ^3H_2O (1 mCi/g) was purchased from New England Nuclear Corp., Boston, MA. ^{14}C-LZP (7.7 or 10.5 μCi/mg) was generously supplied by Ciba-Geigy Corporation, Summit, NJ.

Animals. SD (Sasco Inc., Omaha, NE), WK, and SH (Taconic Farms, Germantown, NY) rats were exposed to a 12-hr light:dark cycle and housed two per cage. Rats were fed with commercial rat chow and tap water ad libitum and were allowed an acclimatization period of at least 4 days prior to experimentation.

Pharmacokinetic Studies with Blood and CSF. Male rats (SH or SD) weighing 300–340 g were kept anesthetized with pentobarbital (50 mg/kg, i.p.) and maintained at 37°C. With the animal on its back, the left femoral artery (for blood collection) and vein (for drug administration) were cannulated with silicone tubing (0.012-in. ID × 0.025-in. OD) containing heparin (100 units/ml). The right femoral artery was isolated (for blood pressure measurement). Then the animal was placed on its stomach and the skull was exposed. A small hole was then drilled by hand using a dental burr on the sagittal midline immediately rostral to the interparietal–occipital bone suture. A 5-cm-long cannula (PE-50) tubing was gently placed in the cisterna magna to a depth of 0.6–0.8 cm. The skull was cleaned of any escaping CSF via the gap at the cannula/skull interface and the gap sealed with epoxy glue (Krazy Glue, Krazy Glue Inc., Itasca, IL). Then the animal was placed on its back again, and CSF flows via the cannula were aided by gravity. This CSF collection method was successful in about 80% (9 of 11 rats) of the attempts made. Blood contamination of CSF (judged visually) was an occasional problem observed in about 20% of the animals; such rats were not used. The right femoral artery (exposed earlier) was then connected to a Physiograph (Model 7,

[1] Schools of Pharmacy and Medicine, University of Missouri, Kansas City, Missouri 64108.
[2] Research Department, Ciba-Geigy Corporation, Summit, New Jersey 07901.
[3] To whom correspondence should be addressed at 2411 Holmes (M3-206), Schools of Pharmacy and Medicine, University of Missouri-Kansas City, Kansas City, Missouri 64108-2792.

0724-8741/92/0200-0236$06.50/0



3-[(5-amino-1-carboxy1S-pentyl)-amino]-2,3,4,5-tetrahydro-2-oxo-3S-1H-benzazepine-1-acetic acid

Fig. 1. Chemical structure of LZP.

Grass Instruments Co., Quincy, MA) via a transducer (Model P23ID, Gould Statham Instruments Inc., Puerto Rico) for the blood pressure determination. The normotensive (SD) group had a MAP of 95 ± 12 mm Hg (range, 83–112 mm Hg); the corresponding value for the SH group was 136 ± 15 mm Hg (range, 118–158 mm Hg), which was significantly higher ($P < 0.05$). Following injection of 3 mg/kg of ^{14}C-LZP via the left femoral vein, a 50:50 mixture of normal saline and 5% glucose was infused (2 ml/hr for the first 2 hr and then reduced to 1 ml/hr). This infusion regimen was found necessary to permit survival of the animal over the duration of the experiment (8–12 hr) and, also, to maintain normal hematocrit (42–46%) and normal blood pressure. Blood (left femoral artery, 50 μl/sample) and CSF (25- to 100-μl aliquots/sample period) were collected over an 8- to 12-hr period. Sampling times for blood were 3, 6, 10, 20, 30, 40, 50, 60, 120, 180, 210, 240, 300, 360, 420, 480, 600, and 720 min. Collection intervals for CSF were 0–15, 15–30, 30–60, 60–90, 90–120, 120–180, 180–240, 240–300, 300–360, and 360–480 min, respectively.

Brain Uptake Studies. Separate groups of SH (MAP, 149 ± 28 mm Hg; $n = 8$) and SD (MAP, 96 ± 11 mm Hg; $n = 7$) rats were used in this part of the study. In addition, WK (MAP, 94 ± 21 mm Hg; $n = 8$) rats, which are genetically more similar to SH rats than the SD species, were used as additional normotensive controls. The method described by Oldendorf (10) was used with minor modifications. The right carotid artery was isolated in rats (250–350 g) under pentobarbital anesthesia (50 mg/kg, i.p.). A 27-G needle was bent at 90° and the tapered end was inserted into the artery, such that carotid blood flow was essentially unchanged. Bleeding was prevented by applying epoxy glue around the artery/needle junction. The drug solution was injected via a 5-cm PE-10 tubing attached to the other end of the needle. A solution (30–35 μl) containing ^{14}C-LZP or ^{14}C-sucrose (for residual correction, discussed below) and a rapidly diffusible reference compound (^3H_2O) was injected via the indwelling cannula. The animal was decapitated 15 sec postinjection [a time sufficient for the bolus to make a single passage through the brain (11)]. The skull was opened and the whole brain was placed on filter paper wetted with cold 0.9% NaCl. Large subarachnoidal and dural blood vessels were removed and discarded. Seven brain regions (medulla, olfactory lobe, colliculus, pons, diencephalon, cortex, and cerebellum) were dissected and analyzed for drug and reference compound by scintillation counting. Since sucrose is an impermeable compound, total-brain radioactivity represents only the amount in the blood associated with the region. Therefore, subtracting (i.e., correction for residual blood) this value from the total tissue drug content provides an estimate of actual drug uptake (10).

Analysis of Tissue, Blood, and CSF. Various brain regions (10–250 mg, accurately weighed) were each dissolved in 2 ml of tissue solubilizer (Protosol, New England Nuclear, Boston, MA) contained in a 22-ml scintillation vial and incubated for about 8–12 hr at 55°C in a shaking water bath. After cooling the vials to room temperature, 10 ml of scintillation fluid (Econofluor, New England Nuclear, Boston, MA) was added to each vial and kept in the dark for 48 hr (to remove chemiluminescence). The vials were then counted for ^{3}H and ^{14}C in a scintillation spectrophotometer (Model LS-230, Beckman Instruments, Fullerton, CA). The counts per minute (cpm) were converted to disintegrations per minute (dpm) using a quench curve obtained by adding known amount of radioactivity to tissues from nontreated animals. Whole blood (50 μl) was added to 0.5 ml of a 2:1 mixture of solubilizer and ethanol contained in a 22-ml scintillation vial and incubated in a shaking water bath for 1 hr at 55°C. After cooling the vial to room temperature, 0.5 ml of 30% hydrogen peroxide was added dropwise, and the vial incubated for an additional 30 min at 55°C. Then, 15 ml of the scintillation fluid (Biofluor, New England Nuclear, Boston, MA) was added to the vial at room temperature, stored for 48 hr, and counted as described earlier for tissues. CSF samples (20–25 μl) were placed in scintillation vials containing 0.5 ml of solubilizer and incubated for 2 hr at 55°C in a shaking water bath. After samples cooled to room temperature, 10 ml of scintillation fluid (Econofluor) was added to the vial and kept in a dark place for 24 hr. Then, the vials were counted for radioactivity. Counts per minute were converted to dpm, a measure of absolute radioactivity, by using predetermined external standard and efficiency curves. The BUI values are calculated by the following equation (10):

$$\text{BUI} = [(^{14}\text{C-brain})/(^3H_2O\text{-brain})]/[^{14}\text{C-mix.}/^3H_2O\text{-mix.}]$$

where ^{14}C-brain and ^3H_2O-brain are the radioactivities of these two compounds in the brain tissue, and ^{14}C-mix and ^3H_2O-mix are the ratios of these two substances in the injection solution.

Data Analysis. LZP blood concentration versus time data obtained after bolus dose administration were analyzed according to standard pharmacokinetic techniques (12). Accordingly, initial parameter estimates were obtained using log concentration–time plots and were then refined by nonlinear regression analysis (13). Area under the concentration–time curve (AUC) was computed by the trapezoidal rule. Volume of the central compartment (V_c), steady-state volume of distribution ($V_{d_{ss}}$), and systemic clearance (Cl) were calculated by the following equations (12):

$$V_c = \text{dose}/C_p^0$$
$$V_{d_{ss}} = \text{dose(AUMC)}/\text{AUC}^2$$
$$\text{Cl} = \text{dose}/\text{AUC}$$

where C_p^0 is the initial blood concentration and AUMC is the first moment of the drug concentration in blood curve obtained by the area under the curve of the plot of the product of concentration and time versus the time from 0 to ∞.

Compartmental behavior was discerned using visual and statistical (Akaike's information criteria and the F test) methods (14,15). Statistical analysis of pharmacokinetic parameters and BUI values consisted of analysis of variance (ANOVA) followed by Duncan's multiple-range test (16) to determine which differences were significant. Values are presented as mean ± SD.

RESULTS

Pharmacokinetic Studies. Blood concentrations of LZP declined in a biexponential manner (Fig. 2) in both the normotensive (predrug MAP, 95 ± 12 mm Hg) and the hypertensive (predrug MAP, 136 ± 15 mm Hg) groups; the predrug MAPs were significantly different ($P < 0.05$). The terminal rate constant for elimination (β), $V_{d_{ss}}$, and CL were similar in these two animal groups (Table I). Other pharmacokinetic parameters [C_p^0, rate constant of distributive phase (α), intercompartment transfer rate constant from the central to the peripheral compartment (k_{12}), intercompartment transfer rate constant from the peripheral to the central compartment (k_{21}), and AUC ratio of CSF to blood concentrations] were significantly ($P < 0.05$) greater in the hypertensive group, except for the volume distribution of the central compartment, V_c, and the $V_c/V_{d_{ss}}$ ratio, which were smaller in SH rats (Table I).

The ratio of CSF to blood drug concentration in SD and SH rats as a function of time is shown in Table II. During the first 30 min, these ratios were significantly higher in the SH group ($P < 0.05$). During the terminal phase (60–480 min) these ratios were, however, quite comparable between SD (range, 0.13–0.29) and SH (0.15–0.32) rats ($P > 0.05$). In SH rats, the peak CSF drug concentration was 1.49 ± 0.78 μg/ml ($n = 5$); the corresponding value for the SD rats was 0.47 ± 0.15 μg/ml ($P < 0.05$, $n = 4$). Following an equilibration period of about 60 min, CSF concentrations declined, approximately in parallel with blood concentrations (Fig. 2).

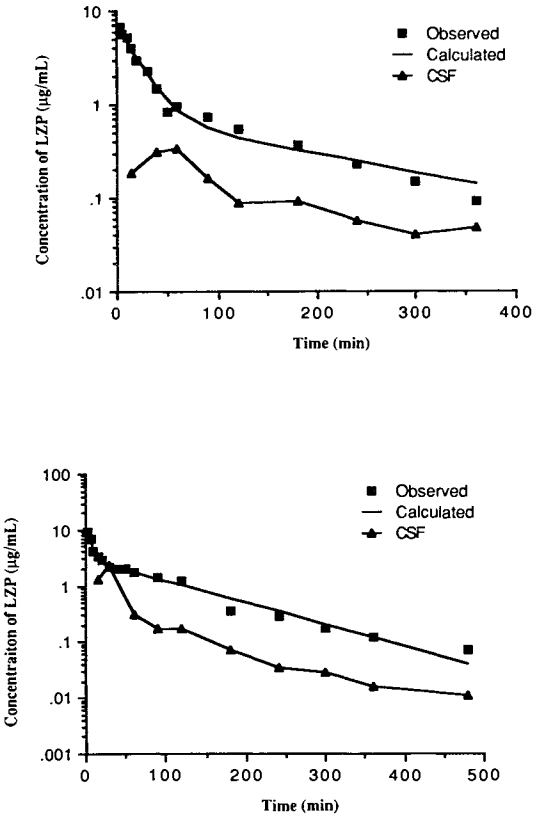


Fig. 2. Experimental blood (■) and CSF (▲) concentrations of LZP following a 3 mg/kg intravenous bolus dose of ^{14}C-LZP in SD (top) and SH (bottom) rats. Lines drawn through blood data are theoretical values based on a biexponential equation.

Drug-induced decreases in blood pressure are shown in Fig. 3. As stated, predrug MAPs were 95 ± 12 and 136 ± 15 mm Hg in SD and SH rats, respectively. Immediately after injection (about 5 min), a steep decline in MAP was observed in both groups. In the 0- to 15-min interval, the drop in MAP values was 23 ± 8 and 60 ± 12 mm Hg ($P < 0.05$) in SD and SH rats, respectively. The hypertensive group exhibited a maximum drop (during the 15- to 30-min interval) of 63 ± 13 mm Hg in MAP and the blood pressure stabilized in about 30 min. In the normotensive (SD) group, the drop in MAP was lower, as might be expected (22 ± 7 mm Hg; also during the 15- to 30-min interval), and the pressure stabilized after about 60 min. As shown (Fig. 3), postdrug MAP values were not significantly different between the two groups of rats after that time ($P > 0.05$).

Brain Uptake Studies. Brain uptake indexes of LZP in the three species of rats are shown in Table III. The reported BUIs have been corrected for residual blood contribution using sucrose as described (10). It was found that only one region (medulla) showed appreciable uptake; no significant differences were observed ($P > 0.05$) between the groups. Uptake indexes in the other six regions were essentially zero across all three species. In contrast to BUI values, corrected tissue drug concentrations in six of the seven brain regions in the hypertensive group were consistently higher ($P < 0.05$) than the corresponding values obtained in the normotensive (SD and WK) groups (Table IV). Similarly, regional concentrations of the marker (^3H_2O) were also significantly ($P < 0.05$) higher in five of the seven regions between SH and WK rats and four of the seven regions between SH and SD rats (Table V). Similarities in regional uptake of LZP were also observed in the three species of rats that were used. With respect to brain LZP uptake, the medulla, olfactory lobe, and diencephalon ranked the highest, the second highest, and the lowest among the seven regions in all three species (Table IV). Within each species, tissue concentrations in the other four regions were essentially equal.

Commercially obtained SH rats exhibited a wide range of MAPs. Therefore, the correlation between brain tissue uptake of LZP and MAP was also examined. A linear trend was observed between regional brain concentrations of LZP and MAP (Fig. 4). However, statistical significance ($P < 0.05$) was obtained only for the medulla, pons, and cortex, with P values of 0.064, 0.27, 0.16, and 0.91 for colliculus, diencephalon, cerebellum, and olfactory lobe, respectively. Similarly, concentrations of ^3H_2O were also related to MAP, with linear relationships being observed for the olfactory lobe, colliculus, pons, and cortex ($P < 0.05$); P values of 0.068, 0.10, and 0.17 were obtained for medulla, diencephalon, and cerebellum, respectively (Fig. 4). For reasons of clarity and space economy, only those regions exhibiting a significant correlation between MAP and uptake of LZP and/or ^3H_2O (except olfactory lobe) are shown.

Table I. Pharmacokinetic Parameters of Libenzapril in Rats*

	MAP (mm Hg)	C_p^o (μg/ml)	α (min^{-1})	β (min^{-1})	k_{12} (min^{-1})	k_{21} (min^{-1})	V_c (ml/kg)	$V_{d_{ss}}$ (ml/kg)	$V_c/V_{d_{ss}}$	CL (ml/min * kg)	$AUC_{(csf/blood)}$
SD											
1	112	7.68	0.051	0.0047	0.0206	0.0092	390	886	0.44	10.1	0.15
2	92	10.22	0.074	0.0052	0.039	0.022	294	455	0.65	5.18	0.055
3	94	12.27	0.060	0.0080	0.0224	0.0158	244	500	0.49	7.7	0.13
4	83	5.97	0.038	0.018	0.0025	0.0214	502	622	0.81	12.0	0.10
Mean	95[a]	9.03[b]	0.055[c]	0.0089	0.021[d]	0.017[e]	357[f]	615	0.59[g]	8.75	0.109[h]
SD	12	2.77	0.015	0.0061	0.014	0.0059	113	193	0.16	2.95	0.041
SH											
5	124	14.7	0.19	0.0087	0.1142	0.0570	203	575	0.35	6.0	0.17
6	128	14.5	0.19	0.0090	0.1164	0.0463	206	663	0.31	7.6	0.22
7	118	12.6	0.15	0.0083	0.0878	0.0348	237	745	0.31	8.5	0.27
8	142	12.2	0.15	0.0029	0.0950	0.0481	245	562	0.43	2.2	0.21
9	158	20.3	0.31	0.0198	0.1765	0.0808	147	592	0.25	11.4	—
Mean	136[a]	14.9[b]	0.198[c]	0.0097	0.1180[d]	0.0531[e]	208[f]	627	0.33[g]	7.1	0.21[h]
SD	15	3.23	0.065	0.0061	0.0349	0.0176	38	76	0.066	3.3	0.041

* Numbers with the same superscript letter significantly different ($P < 0.05$).

DISCUSSION

Libenzapril, a potent angiotensin converting enzyme (ACE) inhibitor, was selected as a model compound, primarily because it is not metabolized and not protein bound (17); hence total radioactivity represents unchanged free drug. In addition, LZP appears to cross the BBB and its antihypertensive effect may be centrally mediated like captopril, another ACE inhibitor (18).

Rats (SH, 16 weeks old) used in this study reach the hypertensive state at 4–5 weeks of age when the systolic pressure can be as high as 150 mm Hg (19). MAP values in rats reported in the literature depend on the species, the artery used for measurement, and the status (awake or unconscious) of the animals (20). In anesthetized rats using the femoral artery (as in this study), a MAP value of 117 ± 11 mm Hg was reported (21). In our studies, MAP values for SD rats were 95 ± 12 mm Hg in the pharmacokinetics study and 96 ± 11 mm Hg in the brain uptake studies; the corresponding values for SH rats (136 ± 15 and 149 ± 28 mm Hg) were significantly greater ($P < 0.05$); WK rats had a MAP of 94 ± 21 mm Hg (Tables I and III). Pentobarbital anesthesia lowered blood pressure, as has also been reported (22). Blood pressure data provided by the supplier (Taconic Farms) in seven SH animals, using the tail cuff method, was 215 ± 10 mm Hg. In the experimental setup (pentobarbital with anesthetized), the corresponding systolic pressure measured in our laboratory was 165 ± 13 mm Hg. In view of the factors (methodical and site) discussed above, estimation of the actual drop in blood pressure due to anesthesia is difficult. Effects of pentobarbital on BBB permeability are unknown. However, since the present investigation is a comparative study, this issue is not a concern.

Pharmacokinetic Studies. Since the animals were used immediately after implantation of cannula in the cisterna magna, blood contamination of CSF is a possibility and was excluded by visual examination; even 1 μl of blood in the volume of CSF collected (25–100 μl) would have been readily apparent. Besides, CSF concentrations of LZP increased during the first 30–60 min, when blood concentrations of the drug declined (Fig. 3). The collection technique (i.e., draining CSF) is physiologically consistent, because CSF is continuously produced and absorbed into blood, (23). Since CSF concentrations of LZP are relatively much lower than blood LZP, CSF collection will have little effect on the

Table II. Ratio of CSF/Blood Concentration of LZP

Time (min)[a]	SH	SD
7.5	0.26 ± 0.039*	0.071 ± 0.027
22.5	0.51 ± 0.31*	0.10 ± 0.007
45	0.27 ± 0.14	0.15 ± 0.08
75	0.29 ± 0.18	0.19 ± 0.06
105	0.20 ± 0.077	0.19 ± 0.07
150	0.22 ± 0.12	0.32 ± 0.23
210	0.19 ± 0.11	0.28 ± 0.05
270	0.23 ± 0.18	0.26 ± 0.007
330	0.25 ± 0.26	0.26 ± 0.014
420	0.13 ± 0.035	0.26 ± 0.18

[a] Midpoint of CSF collection interval.
* Significantly different ($P < 0.05$) from the corresponding values in the SD group.

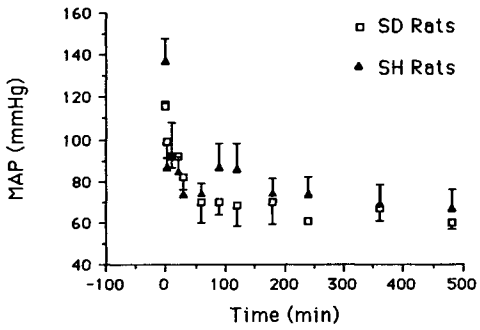


Fig. 3. Effect of LZP on the time course of MAP in SD and SH rats. The test drug (3 mg/Kg) was administered iv. Values are mean ± SD; $n = 4$ in normotensive (SD) and $n = 5$ in SH rats.

Table III. Brain Uptake Index[a] of Libenzapril[b] in Rats*

Region	SD	WK	SH
Medulla	0.190 ± 0.11	0.11 ± 0.057	0.172 ± 0.049
Olfactory lobe	0.0016 ± 0.0023	0.039 ± 0.023	0.032 ± 0.029
Colliculus	0.0082 ± 0.012	0.044 ± 0.044	0.0078 ± 0.022
Pons	0.031 ± 0.023	0.019 ± 0.029	0.029 ± 0.019
Diencephalon	0.0067 ± 0.016	0.019 ± 0.024	**
Cortex	**	0.0074 ± 0.011	0.0031 ± 0.0055
Cerebellum	0.040 ± 0.011	0.034 ± 0.022	0.0013 ± 0.0021
MAP (mm Hg)	96 ± 11[a]	94 ± 21[b]	149 ± 28[a,b]
N	7	8	8

[a] Data after correction for blood residue.
[b] 3 mg/kg.
* Numbers with the same superscript letter significantly different ($P < 0.05$).
** Indeterminate (negative value).

blood and CSF kinetics of the drug. Therefore, artifactual effects due to blood contamination of CSF or the sampling technique used are unlikely.

The decline of blood LZP in SH and SD rats as a function of time was best described by a biexponential equation ($C_p = A * e^{-\alpha * t} + B * e^{-\beta * t}$), characteristic of the two-compartment model, which consists of rapid and slow equilibrating regions (Table I). A high degree of confidence in the estimated parameter was observed. The *r* values for these regressions ranged from 0.97 to 0.99. With one exception, parameter coefficients of variation were small (4–38%) in both SH and SD rats. In addition, 95% confidence intervals were narrow except in the case noted (SD 4) where a high coefficient of variation (6–282%) was observed and the 95% confidence interval included the value of zero. Chronic hypertension influenced the disposition of LZP. While systemic clearances (CL = dose/AUC) and steady-state volume of distribution ($V_{d_{ss}}$) were similar between SH and SD rats, significantly higher α, k_{12}, and k_{21} values were observed in SH rats ($P < 0.05$). This indicated that LZP equilibrated more rapidly in the hypertensive rats and is consistent with intuitive expectations (better mixing due to pressure). The reason for smaller V_c in SH rats is opened to speculation. As shown Fig. 3, blood pressure in SH and SD rats became essentially identical after about 1 hr. Therefore, the lack of differences in $V_{d_{ss}}$ (calculated based on 12-hr data) may be due to the antihypertensive effect of LZP. A drug with a relatively small $V_c/V_{d_{ss}}$ ratio may show an unusually large peak-to-trough concentration ratio over a dosing interval even when administered relatively frequently (12). Since the $V_c/V_{d_{ss}}$ ratio was significantly lower in SH rats, it suggests that chronic hypertensive patients may experience a wider range of plasma concentrations. Dosage adjustment may therefore be more critical for drugs with narrow therapy ranges in such patients.

Collins and Dedrick (24), using theoretical simulations, demonstrated that an increase in brain capillary permeability of a drug should cause an increase in the AUC ratio of CSF to blood as well as their concentration ratio during the terminal phase when both CSF and blood concentrations have a parallel decline. In the present study, the uptake of LZP into the brain was measured by both the ratio of CSF to blood AUCs (Table I) and the time-dependent ratio of CSF to blood concentration (Table II). A significantly ($P < 0.05$) larger AUC was observed in the hypertensive rats (Table I). The CSF-to-blood concentration ratios were also higher during the first 75 min (when the blood pressure was significantly greater in the SH group), although significant difference in this ratio was obtained only during the first two collection intervals (0–15 and 15–30 min). During the terminal phase this ratio was similar in both groups except for the last sample, which approached background radioactivity and was subject to large analytical error. An explanation for the terminal CSF/blood concentration ratio to be essentially the

Table IV. Brain Concentration[a] of Libenzapril[b] in Rats*

Region	SD	WK	SH
Medulla	0.21 ± 0.096[a]	0.30 ± 0.16[b]	0.70 ± 0.46[a,b]
Olfactory lobe	0.091 ± 0.052[c]	0.22 ± 0.11[d]	0.63 ± 0.22[c,d]
Colliculus	0.036 ± 0.026[e]	0.041 ± 0.009[f]	0.21 ± 0.16[e,f]
Pons	0.046 ± 0.016[g]	0.067 ± 0.042[i]	0.19 ± 0.11[g,i]
Diencephalon	0.019 ± 0.018	0.007 ± 0.011	0.13 ± 0.11
Cortex	0.046 ± 0.023[j]	0.072 ± 0.045[k]	0.19 ± 0.150[j,k]
Cerebellum	0.067 ± 0.022[l]	0.078 ± 0.044[m]	0.21 ± 0.150[l,m]
MAP (mm Hg)	96 ± 11[n]	94 ± 21[p]	149 ± 28[n,p]
N	7	8	8

[a] 3 mg/kg.
[b] ng/mg tissue/mg dose.
* Numbers with the same superscript letter significantly different ($P < 0.05$).

Table V. Brain ^3H_2O in Normotensive and Hypertensive Rats*

Region	^3H_2O (μg/mg tissue)		
	SD	WK	SH
Medulla	0.099 ± 0.040	0.11 ± 0.024	0.15 ± 0.034
Olfactory lobe	0.25 ± 0.11[a]	0.25 ± 0.15[b]	0.71 ± 0.53[a,b]
Colliculus	0.60 ± 0.30[c]	0.28 ± 0.30[d]	1.20 ± 0.58[c,d]
Pons	0.088 ± 0.042	0.095 ± 0.027	0.13 ± 0.041
Diencephalon	0.47 ± 0.14[e]	0.32 ± 0.30[f]	1.06 ± 0.57[e,f]
Cortex	0.40 ± 0.034	0.33 ± 0.23[g]	0.79 ± 0.48[g]
Cerebellum	0.13 ± 0.042[h]	0.099 ± 0.037[i]	0.35 ± 0.21[h,i]

* Numbers with the same superscript letter significantly different ($P < 0.05$).

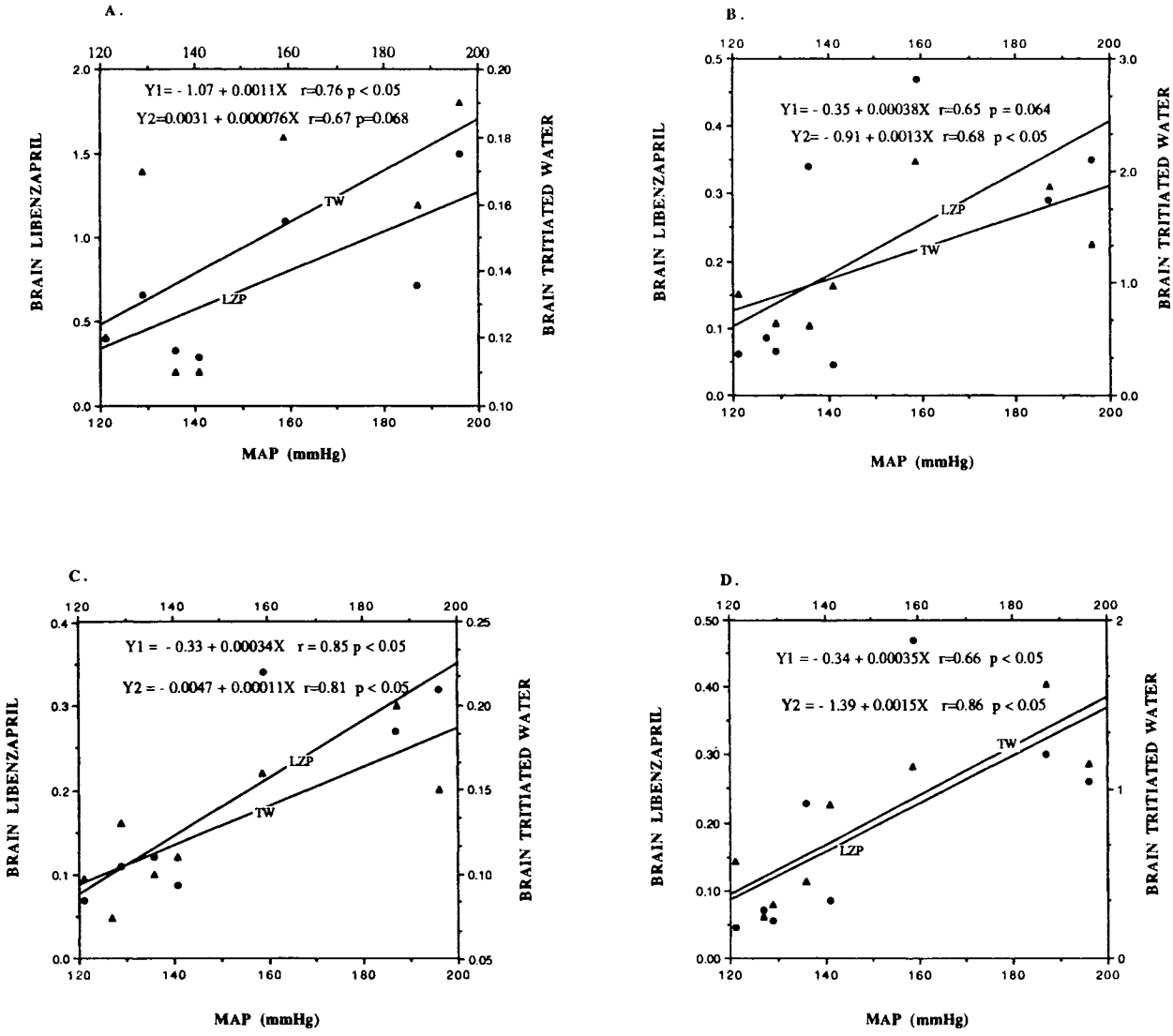


Fig. 4. Effect of MAP on brain tissue concentrations of LZP (●; ng/mg tissue/mg dose) and ^3H_2O (▲; μg/mg tissue) in SH rats. Lines (Y1 for LZP and Y2 for ^3H_2O) were drawn by linear regression. (A) Medulla; (B) colliculus; (C) pons; (D) cortex. TW = ^3H_2O.

same in SD and SH rats can be obtained by examining the pharmacodynamic results. Blood pressure declined rapidly (~5 min) after intravenous bolus administration in both groups but remained significantly higher in SH rats during the first 60 min postadministration; MAP values in SD and SH rats were similar after that time (Fig. 3). It, therefore, appears that enhanced permeability of LZP observed previously (when MAPs were higher) in SH rats was reversed to that of the normotensive group, when blood pressure differences did not exist. Increased brain permeability of LZP in chronic hypertension was also demonstrated in the acute brain uptake study discussed below.

Brain Uptake Index Studies. Brain uptake of LZP was determined 15 sec [time required for single passage through the brain (11)] after intracarotid drug administration. The antihypertensive effect of LZP was not manifested until about 5 min postadministration (Fig. 3). Therefore, BUI values were obtained at predrug MAP.

BUI values (Table III) showed that the brain uptake of LZP, except for the medulla (17–19%), is small (less than 1–3% in the other six regions examined) during a single passage through the brain. The highest BUI in medulla was unexpected because medulla is not directly supplied by the internal carotid artery; the basilar artery provides most of the blood supply. However, the injected drug can reach the medulla via the circle of Willis because the pressure of injection alters the blood-flow pattern transiently (25). The preferential uptake of LZP by the medulla is speculated to be due to regional differences in capillary density. The poor overall brain permeability of LZP is best explained by its poor lipophilicity; its chloroform/phosphate buffer (pH 7.4) partition coefficient is <0.01 (unpublished observations).

In this investigation, brain uptake of the drug and ^3H_2O [classical reference marker (11)] was found to be essentially identical in SD and WK rats. Therefore, either strain of rats can be used as normotensive controls for the SH rats. When a drug is injected via the common carotid artery, only about 10–20% reaches the brain. A reference marker is used along with the drug in BUI studies primarily to correct for this variability. Results from this study showed that the uptake of both ^{14}C-LZP and ^3H_2O in the hypertensive group was two to five times higher than that in the normotensive groups (Tables IV and V). In this connection it is interesting to note that tyrosine uptake is enhanced in SH rats (26). This increased uptake of LZP could be either due to increased BBB permeability in hypertension or due to differences in cerebral blood flow. Blood flow, however, in SH and WK rats is similar (27–30). In addition, the brain permeability is relatively blood flow independent when the BUI value is less than 30% (31). A linear relationship (three of the seven regions were statistically significant; the P values of colliculus, diencephalon, cerebellum, and olfactory lobe were 0.064, 0.27, 0.16, and 0.91, respectively) was also observed between MAP and regional brain LZP and ^3H_2O (Fig. 4) concentration. Therefore, results from brain uptake studies, which are consistent with the CSF data, showed a higher blood–CNS permeability of LZP in hypertension. Because of increased uptake of both LZP and ^3H_2O in hypertension, BUI values (Table III) did not reflect increased brain uptake of LZP in SH rats (Table IV and Fig. 4).

In summary, very few data are currently available on the effect of chronic hypertension on the blood–brain barrier transport of drugs, which are in most cases of low molecular weight. Based on our results with LZP, chronic hypertension can increase the BBB permeability of such small polar molecules. Mechanisms responsible for increased permeability of LZP in chronic hypertension need to be elucidated. It also appears that such disruption of BBB in this model of chronic hypertension is reversible when systemic pressure is reduced to the normal level. Results of the present study suggest that dosage requirements may be lower in chronic hypertensive patients, particularly for drugs which act on the CNS. Further studies are needed to evaluate this phenomenon and its clinical significance.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Cynthia Nyquist-Battie for advice on brain dissection and Dr. Gregory Kochak for helpful comments on data analysis. Financial support from Ciba-Geigy Corporation to S. Melethil and UMKC Women's Council (Gracia Bremer Award) to Jianping Tang is also acknowledged.

REFERENCES

1. B. B. Johansson, C. L. Li, Y. Olsson, and I. Klatzo. The effect of acute arterial hypertension on the blood-brain barrier to protein tracers. *Acta Neuropathol. (Berl.)* 16:117–124 (1970).
2. B. B. Johansson. Effect of an acute increase in intravascular pressure on the blood-brain barrier. A comparison between conscious and anesthetized rats. *Stroke* 9:588–590 (1978).
3. W. G. Mayhan, F. M. Faraci, and D. D. Heistad. Mechanisms of protection of the blood-brain barrier during acute hypertension in chronically hypertensive rats. *Hypertension* 9 (Suppl. III):101–105 (1987).
4. A. H. Werber and M. C. Fitch-Burke. Effect of chronic hypertension on acute hypertensive disruption of the blood-brain barrier in rats. *Hypertension* 12:549–555 (1988).
5. S. M. Mueller and D. D. Heistad. Effect of chronic hypertension on the blood-brain barrier. *Hypertension* 2:809–812 (1980).
6. R. A. Majack and R. C. Bhalla. Ultrastructural characteristics of endothelial permeability pathways in chronic hypertension. *Hypertension* 3:586–595 (1981).
7. K. J. Isselbacher, R. D. Adams, E. Braunwald, R. G. Petersdorf, and J. D. Wilson. *Principles of Internal Medicine,* McGraw–Hill, New York, 1980.
8. K. Okamoto and K. Aoki. Development of a strain of spontaneously hypertensive rats. *Jap. Circ. J.* 27:282–294 (1963).
9. C. C. Chiueh and J. J. Kopin. Hyperresponsivity of spontaneously hypertensive rats to indirect measurement of blood pressure. *Am. J. Physiol.* 234:H690–H695 (1978).
10. W. H. Oldendorf. Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. *Am. J. Physiol.* 221:1629–1639 (1971).
11. W. H. Oldendorf. Brain uptake of metabolites and drugs following carotid arterial injections. *Trans. Am. Neurol. Assoc.* 96:46–50 (1971).
12. M. Gibaldi and D. Perrier. *Pharmacokinetics,* Marcel Dekker, New York, 1982, pp. 2–84.
13. C. M. Metzler and D. L. Weiner. *PCNONLIN (V3.0) User's Guide,* Statistical Consultants, Inc., Lexington, KY, 1989.
14. K. Yamaoka, T. Nakagawa and T. Uno. Application of Akaike's Information Criterion (AIC) in the evaluation of linear pharmacokinetic equations. *J. Pharmacokin. Biopharm.* 6:165–175 (1978).
15. H. G. Boxenbaum, S. Riegelman, and R. M. Elashofft. Statistical estimations in pharmacokinetics. *J. Pharmacokin. Biopharm.* 2:123–147 (1974).

16. SAS Institute. *SAS User's Guide,* SAS Institute, Raleigh, NC, 1979, pp. 237–241.
17. G. M. Kochak, R. L. Choi, J. K. De-Silva, and P. Reydel-Bax. Physiological disposition of CGS 16617 in rat, dog, and man. *Drug Metab. Disposit.* 17:669–672 (1989).
18. J. A. Norman, M. Lehman, F. R. Goodman, B. W. Barclay, and M. B. Zimmerman. Central and peripheral inhibition of angiotensin converting enzyme (ACE) in the SHR: Correlation with the antihypertensive activity of ACE inhibitors. *Clin. Exp. Hypertens.* [*A*] 9:461–468 (1987).
19. Tocanic Farms laboratory animal and services. Research report (1990).
20. C. Petty. *Research Techniques in the Rat,* C. C. Thomas, Springfield, IL, 1982, p. 35.
21. E. R. Krieger. Neurogenic hypertension in the rat. *Circ. Res.* 15:511–521 (1964).
22. S. K. Wixson, W. J. White, H. C. Hughes, Jr., C. M. Lang, and W. K. Marshall. The effects of pentobarbital, fentanyl-droperidol, ketamine-xylazine and ketamine-diazepam on arterial blood pH, blood gases, MAP and heart rate in adult male rats. *Lab. Anim. Sci.* 37:736–742 (1987).
23. A. C. Guyton. The special fluid system of the body. In *Textbook of Medical Physiology,* Saunders, Philadelphia, PA. 1986, pp. 374–375.
24. J. M. Collins, and R. L. Dedrick. Distributed model for drug delivery to CSF and brain tissue. *Am. J. Physiol.* 245:R303–R310 (1983).
25. L. A. Wade and R. Katzman. Rat brain regional uptake and decarboxylation of 1-DOPA following carotid injection. *Am. J. Physiol.* 228:352–359 (1975).
26. P. Hatzinikolaou, P. Brecher, and H. Gavras. Chronic hypertension increases tyrosine transport across the BBB in the rat. *Life Sci.* 29:1657–1660 (1981).
27. S. Strandgaard, J. Olsen, E. Skinhoj, and N. A. Lassen. Autoregulation of brain circulation in severe arterial hypertension. *Br. Med. J.* 1:507–510 (1973).
28. W. E. Hoffman, R. F. Alberecht, and D. J. Miletich. Nitroglycerin induced hypotension will maintain CSF in hypertensive rats. *Stroke* 13:225–228 (1982).
29. S. Sadoshima, D. W. Busija, and D. D. Heistad. Mechanisms of protection against stroke in stroke-prone spontaneously hypertensive rats. *Am. J. Physiol.* 224:H406–H412 (1983).
30. B. B. Johanson. Cerebral vascular bed in hypertension and consequences for the brain. *Hypertension* 6 (Suppl. III):81–86 (1984).
31. M. W. B. Bradbury, C. S. Patlak, and W. H. Oldendorf. Analysis of brain uptake and loss of radiotracers after intracarotid injection. *Am. J. Physiol.* 229:1110–1115 (1975).