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# INCREASED BLOOD-BRAIN BARRIER PERMEABILITY OF AMINO ACIDS IN CHRONIC HYPERTENSION

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Abstract A previous communication from this laboratory reported that brain uptake of libenzapril, a small polar molecule, was enhanced in chronic hypertension (1). The objective of this investigation was to determine if this was a more generalized phenomenon. Therefore, experiments were undertaken to examine the effect of chronic hypertension on the brain uptake of tryptophan (an amino acid with high brain permeability) and glutamic acid (one with low permeability). Brain concentrations of these two amino acids were 5- to 12-fold greater in chronic hypertensive rats, as compared to normotensive rats; the corresponding brain uptake index (BUI) values were 2- to 5-fold higher in the former group. Since blood-brain barrier transport of amino acids involve both saturable (carrier) and non-saturable (most likely, diffusion via pores) mechanisms, data from this study show that hypertension can enhance BBB transport of amino acids by affecting one or both of these pathways.

## Introduction

Very little is known about the effect of chronic hypertension on the blood-brain barrier (BBB) permeability of small (M.W. < 500) polar molecules. Previous studies from our laboratory (1) showed that chronic hypertension increased brain uptake of libenzapril (LZP), a small polar molecule with a lysine side-chain. It has also been reported that BUI of tyrosine increased in chronic hypertension (2). Therefore, the objective of this study was to determine if this was a more generalized phenomenon and applied to other small molecules. As a first step in this direction, the study was extended to include two other amino acids, namely tryptophan and glutamic acid, which represent high and low permeable compound, respectively.

## Materials and Methods

**Radioactivity Materials:** $^{3}H_2O$ (1 mCi/gm), $^{14}C$-tryptophan (54.8 mCi/mmol) and $^{14}C$-glutamic acid (293.3 mCi/mmol) were purchased from New England Nuclear Corp., Boston, MA.

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**Animals:** Sprague-Dawley, SD (Sasco Inc., Omaha, NE) and spontaneously hypertensive, SH (Taconic Farms, Germantown, NY) rats were exposed to 12-hour 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.

**Experimental:** Male normotensive (SD) or chronic hypertensive (SH) rats weighing 280-330 gms were kept anesthetized with pentobarbital (50 mg/kg, i. p.) and maintained at 37 °C. The right femoral arteries were cannulated for blood pressure measurement. The BUI method, as reported by Oldendorf (3) with minor modifications (1), was then used to examine brain permeability (ipsilateral side) of tryptophan and glutamic acid. Briefly, the right carotid artery was isolated. 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. Following the phenylephrine infusion, the drug solution ($^{14}$C-LZP, 3 mg/Kg) along with a rapidly diffusible reference compound ($^{3}H_2O$) was injected via the indwelling carotid cannula. The animal was decapitated 15 sec post injection. Four brain regions, namely cerebellum, medulla and pons, cortex and a composite region consisting of the remainder of the brain were dissected and analyzed for $^{14}$C-amino acid and reference ($^{3}H_2O$) compound by scintillation counting. Brain uptake index values were calculated by the relationship: BUI = ($^{14}$C amino acid in brain / $^{3}H_2O$ in brain) / ($^{14}$C-amino acid in injected solution / $^{3}H_2O$ in injected solution). Tissue amino acid concentrations were corrected for residual blood (1). Regional blood volume were determined using sucrose as a non-diffusible marker (3).

**Data Analysis:** All values are presented as mean ± SD, n = 6 - 7. Regional BUI and brain concentrations (dpm/mg tissue) of amino acids were tested for differences by using the unpaired t test (4). Outliers were dropped using the Dixon's test for extreme values (5).

## Results

Mean arterial pressures (MAP) for the two SD groups were 99 ± 12 and 100 ± 15 mmHg respectively; the corresponding MAPs for the SH groups were 183 ± 9.0 and 178 ± 30 mmHg respectively. Mean MAPs in the SH rats are significantly ($p < 0.05$) higher than those in the SD ra (Table I). Regional BUI values for tryptophan and glutamic acid are shown in Table I. As shown BUI values in SH rats were greater about 2- to 3-fold for tryptophan in all 4 regions and 3- to 5-fo for glutamic acid in all regions except the medulla and pons. In the tryptophan group, differenc were significant ($p < 0.05$) for all 4 regions examined; in the glutamic acid group, only (cerebellum, cortex and composite) out of the 4 regions were significant ($p < 0.05$). Regional bra concentrations (dpm/mg tissue) of $^{14}$C-tryptophan and $^{14}$C-glutamic acid are shown in Table II. SH rats, these values were significantly ($p < 0.05$) increased in all 4 regions except the medulla a pons; the enhancement was about 5- to 6-fold for tryptophan and 6- to 12-fold for glutamic ac excluding data for medulla and pons. Brain concentrations of glutamic acid and tryptophan in S rats in this pooled region increased only about 20 and 60%, respectively, and these increases we not significantly different from the corresponding values in SD rats.

## Discussion

A previous study from our laboratory (1) showed no differences in the BBB permeability of LZ between the two genetic strains of normotensive (SD and Wistar Kyoto) rats. Data from the prese study were also comparable to values reported by Oldendorf in Wistar rats (6). Brain uptake ind values for tryptophan for SD rats in this study were in good agreement with the value of 0. (whole brain) reported by Oldendorf; for glutamic acid, BUI values for two regions (cortex a composite) were in reasonable agreement with the reported (6) values (0.032, whole brai Therefore, the greater brain uptake of amino acids in SH rats observed in the present study unlikely to be due to strain differences.

Results from this study showed that brain uptake (indicated by both BUI and brain concentration) tryptophan and glutamic acid was significantly greater in chronic hypertensive (SH) rats. Bloc

brain barrier transport of tryptophan and glutamic acid involve both saturable (carrier) and non-saturable (most likely, diffusion via pores) mechanisms (6, 7). Higher BUI and brain concentration

**TABLE I**

**Brain Uptake Index of Tryptophan and Glutamic Acid in Rats**

| | Tryptophan* | | Glutamic Acid** | |
|---|---|---|---|---|
| **Region** | SD | SH | SD | SH |
| **Cerebellum** | $0.22 \pm 0.044^{a}$ | $0.64 \pm 0.15^{a}$ | $0.15 \pm 0.041^{e}$ | $0.42 \pm 0.11^{e}$ |
| **Medulla + Pons** | $0.24 \pm 0.072^{b}$ | $0.41 \pm 0.10^{b}$ | $0.42 \pm 0.11$ | $0.52 \pm 0.22$ |
| **Cortex** | $0.29 \pm 0.050^{c}$ | $0.78 \pm 0.065^{c}$ | $0.077 \pm 0.010^{f}$ | $0.39 \pm 0.13^{f}$ |
| **Composite** | $0.27 \pm 0.046^{d}$ | $0.72 \pm 0.067^{d}$ | $0.078 \pm 0.017^{g}$ | $0.35 \pm 0.089^{g}$ |
| MAP (mmHg) | $99 \pm 12^{g}$ | $183 \pm 9.0^{g}$ | $100 \pm 15^{h}$ | $178 \pm 30^{h}$ |
| n | 7 | 7 | 6 | 7 |

*: 0.117 μM/kg; **: 0.0907 μM/kg
Numbers with same superscript indicate significant difference ($p < 0.05$)

**TABLE II**

**Regional Brain Concentration* of $^{14}$C-Tryptophan and $^{14}$C-Glutamic Acid in Rats**

| | Tryptophan | | Glutamic Acid | |
|---|---|---|---|---|
| **Region** | SD | SH | SD | SH |
| **Cerebellum** | $21.4 \pm 9.92^{a}$ | $110 \pm 98.5^{a}$ | $17.3 \pm 5.92^{d}$ | $109 \pm 45.8^{d}$ |
| **Medulla + Pons** | $10.4 \pm 1.91$ | $16.7 \pm 4.87$ | $33.0 \pm 16.5$ | $39.7 \pm 20.4$ |
| **Cortex** | $71.8 \pm 55.9^{b}$ | $417 \pm 128^{b}$ | $25.7 \pm 11.6^{e}$ | $312 \pm 160^{e}$ |
| **Composite** | $76.0 \pm 54.2^{c}$ | $372 \pm 58.0^{c}$ | $26.3 \pm 8.25^{f}$ | $268 \pm 13.0^{f}$ |

*: dpm/mg tissue
Numbers with same superscript indicate significant difference ($p < 0.05$)

of these amino acids in SH rats indicated that chronic hypertension can enhance these transport mechanisms (e. g. up-regulation of amino acid transporter and/or pressure-induced opening of pores). The extent of increase for glutamic acid (about 6- to 12-fold increase) was greater than that (5- to 6-fold) for tryptophan. Since glutamic acid is more polar than tryptophan, greater brain uptake of the former in SH rats suggests that the opening of pressure-sensitive channels (or pores) may predominate over carrier up-regulation in chronic hypertension.

Increases in brain concentrations of tryptophan and glutamic acid (5- to 12-fold) in hypertensive rats were much greater than the corresponding BUI values (2- to 5-fold). This is due to the fact that

concentration of $^3H_2O$ (the reference marker) also increased about 3- to 6-fold in SH rats (data not shown). Brain uptake index, expressed as the ratio of brain concentration of the test compound to that of the reference marker ($^3H_2O$), will increase to the same extent as brain concentrations only if the reference marker uptake remains unaltered. In this regard, it was found previously that brain uptake of LZP was significantly increased in chronic hypertension (1), but the corresponding BUI values did not increase because uptake of LZP and $^3H_2O$ increased to the same extent. Though LZP has a lysine side chain, brain uptake of LZP was unaffected in the presence of lysine (unpublished data). Therefore, it appears that increased uptake of both compounds (LZP and $^3H_2O$) in hypertension was mediated via the same mechanism (i.e. diffusion through pressure-sensitive channels (or pores) in the endothelial cells (8). As noted, tryptophan and glutamic acid are transported across BBB via saturable and non-saturable systems. Therefore, if hypertension enhances both pathways, then increases in brain amino acid concentrations should be higher than those of $^3H_2O$ and cause BUI values to be higher as was observed in this study.

Tryptophan and glutamic acid were chosen for this study also because they are precursors of neurotransmitters (serotonin and γ-aminobutyric acid, respectively). Since the synthesis of neurotransmitters is limited by the availability of these precursors, especially that of tryptophan, it is possible that increases in brain uptake of tryptophan and glutamic acid in chronic hypertension can influence the brain synthesis of these neurotransmitters resulting in altered brain function. Further efforts are also needed to identify the molecular basis of the altered BBB amino acid transport in chronic hypertension.

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