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Evidence for Uptake of Nonceruloplasminic Copper in the Brain: Effect of Ionic Copper and Amino Acids (40152)

JERRY G. CHUTKOW

Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Copper (Cu) in the brain is derived from both the ceruloplasminic and the nonceruloplasminic fractions of plasma (1, 2). Regardless of its origin, Cu presumably enters the parenchymal central nervous system either by crossing the endothelial cells of the cerebral capillaries ("blood-brain barrier") or from the ventriculosubarachnoid fluid, by penetrating the epithelial cells of the choroid plexi or by both means. Currently, nothing is known about the mechanisms regulating the exchange of Cu between blood and the central nervous system.

In mammals, Cu is present in several forms in plasma. Depending on the species, from 56 to 99% of the total is tightly incorporated into ceruloplasmin (3). The remaining nonceruloplasminic Cu does not exchange with ceruloplasminic Cu in vivo or in vitro at physiologic pH, except in the presence of *p*-phenylenediamine (4); is presumably in the divalent state [Cu(II)]; and is distributed among three pools that are in equilibrium: Cu loosely bound to albumin (about 90%), Cu complexed to amino acids (about 10%), and Cu in the ionic state (5, 6).

The data in this report concern the influx of nonceruloplasminic Cu(II) into the brain. Answers were sought to three questions: Does the brain retain Cu(II) after a single circulatory pass? If so, what effect does Cu(II) have on its own uptake? Finally, will certain amino acids increase the uptake of Cu(II) as they do in other mammalian cells (7, 8)?

Materials and methods. Animals. Male albino rats (Sprague–Dawley strain) weighing 225–300 g and fed a stock laboratory chow were anesthetized with pentobarbital before all surgical procedures.

Brain uptake index. The net uptake of radiocopper [^{67}Cu(II)] compared with that of tritiated water during a single passage through the microcirculation of the telencephalon–diencephalon was measured 15 sec after injection into the right common carotid system by the method of Oldendorf (9, 10). The injectates containing the radioisotopes were prepared using either Ringer's solution buffered with 4 mmole of *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid buffer or fresh rat serum equilibrated with 5% CO_2 and 95% O_2 as the diluent. Each milliliter of diluent contained 6.3 μCi of tritiated water (^{3}HOH), 3 or 5 μCi of carrier-free ^{67}Cu(II) (Atomic Energy Commission, Oak Ridge, TN), selected amounts of carrier Cu(II) (as $CuCl_2$) and, when indicated, 0.1 μmol of an amino acid.

The warmed (37°) test solution (0.2 ml) was injected directly into the right carotid system in about 1 sec. Fifteen seconds after the onset of the injection, the rat was decapitated; the right cerebrum and hemidiencephalon were dissected free; and duplicate samples of tissue (200–250 mg) were extruded from a 3 ml syringe into separate scintillation vials containing 2.0 ml of tissue solubilizer (Soluene, Packard). Duplicate aliquots (two or three drops) of the injectate, taken directly from the injecting syringe, were transferred to separate vials, each containing solubilizer and 200–250 mg of nonradioactive rat brain.

The samples and aliquots of injectate were analyzed for ^{67}Cu on a gamma counter (Beckman Model 310) at a counting error of less than ±3% and corrected for radioactive decay to a common time. The samples were allowed to stand until the ^{67}Cu decayed to less than 1% of the activity at the time of injection and, after the addition of 10 ml of liquid scintillation solution (Dimilume, Packard), were reassayed for the beta activity of tritiated water in a liquid scintillation counter with an automatic external standard (Tricarb Model 3390, Packard) at a counting error of less than ±1%. No significant contribution from decay of residual ^{67}Cu was detected in any study nor was there any detectable loss of ^{3}HOH during the waiting period. The rate of disintegration of ^{3}HOH per min (dpm) was

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tope to the endothelial cells. Therefore, through a concentration of 1000 ng/ml, one is justified in proposing the existence of an unsaturable carrier for the influx of Cu(II) into or across the cerebral cells. In this regard, these data are similar to those obtained from perfused liver experiments in dogs (12). At a minimum, the cation probably penetrates the endothelial cells; however, further studies will be necessary to determine whether the Cu(II) or a Cu(II)-amino acid complex actually enters the parenchymal interstitial fluid of the brain.

The BUI at 10 ng/ml raises the possibility of a second, saturable carrier operating at very low concentrations of Cu(II). Several additional measurements of BUI between 3.2 and 15 ng/ml would be desirable; because a net BUI of about 16% in six rats at 10 ng/ml, in the absence of any added amino acid, and a value of 5% at 20 ng/ml may be indicative of a carrier which is unsaturated at the former and saturated at the latter level. Studies designed to obtain such data have been deferred, because of the current limited availability and expense of ^{67}Cu.

Clearly, amino acids have a role in the retention of Cu(II) in the brain. Histidine and cystine are the principal microligands of Cu(II) in plasma, at least in man (5, 6); and histidine augments the uptake of Cu(II) into liver, kidney, and neoplastic cells *in vitro* (7, 8). It is surprising, therefore, that histidine did not enhance the BUI of Cu(II) *in vivo*, while several other amino acids did, some considerably so. Tyrosine also was ineffective, indicating a degree of chemical specificity of the Cu-amino acid interaction. Finally, increased entry or, conceivably, retention of Cu(II) does not correlate either with the reported values of BUI for the amino acids (9) or with the ability of the acids to compete with albumin for binding of Cu(II) *in vitro* (6). The meaning of these results and their relationship to the regulation of the exchange of Cu(II) across the blood-brain barrier will have to be clarified in future experiments designed to measure the concentrational effects of each amino acid on Cu(II) uptake and, conversely, of Cu(II) on the BUI of each amino acid.

Summary. The initial net uptake of Cu(II) into the brain was studied in rats using the single microcirculatory pass technique of Oldendorf. The results indicate that the net influx of Cu(II) is facilitated by a nonsaturable process over a wide range of concentrations. Several amino acids enhance the uptake or early retention of Cu(II). However, the amino acids which promote Cu uptake in brain appear to be different from those previously shown to be effective in other cell systems *in vitro*.

1. Marceau, N., and Aspin, N., Amer. J. Physiol. **222,** 106 (1972).
2. Terao, T., and Owen, C. A., Jr., Mayo Clin. Proc. **49,** 376 (1974).
3. Evans, G. W., and Wiederander, R. E., Amer. J. Physiol. **213,** 1183 (1967).
4. Owen, C. A., Jr., Proc. Soc. Exp. Biol. Med. **149,** 681 (1975).
5. Lau, S. J., and Sarkar, B., J. Biol. Chem. **246,** 5938 (1971).
6. Neumann, P. Z., and Sass-Kortsak, A., J. Clin. Invest. **46,** 646 (1967).
7. Harris, D. I. M., and Sass-Kortsak, A., J. Clin. Invest. **46,** 659 (1967).
8. Neumann, P. Z., and Silverberg, M., Nature (London) **210,** 414 (1966).
9. Oldendorf, W. H., Amer. J. Physiol. **221,** 1629 (1971).
10. Chutkow, J. G., *Exp. Neurol.* (in press.)
11. Bradbury, M. W. B., Patlak, C. S., and Oldendorf, W. H., Amer. J. Physiol. **229,** 1110 (1975).
12. Goresky, C. A., Holmes, T. H., and Sass-Kortsak, A., Can. J. Physiol. Pharmacol. **46,** 771 (1968).
13. Oldendorf, W. H., Amer. J. Physiol. **224,** 967 (1973).

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