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IRON, COPPER, AND ZINC IN RAT BRAIN

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Data are presented for the concentrations of total iron, copper and zinc in rat brain. The average values were 227, 39, and 198 nmoles per g brain wet weight, respectively. The importance of exsanguination for brain iron studies is stressed.

The content of iron and copper increased with the age of the animal, while the zinc content decreased slightly. Administration of lysergic acid diethylamide, tremorine dihydrochloride, 2,2′,2″-tripyridine, or 3-acetylpyridine to rats caused no changes in the amounts of the three metals in the brain.

Iron Copper Zinc Brain, rat Rat brain

1. INTRODUCTION

Among the trace metals in brain, iron, copper, and zinc are of special interest since they occur in the same concentration range, and have interesting regional distributions (Spatz, 1922; Warren, Earl and Thompson, 1960; Maske, 1955). Several recent studies have dealt with trace metal levels in the brains of animals treated with various drugs. Hadžović and coworkers (1965, 1966, 1967) found that in rats both tremorine and lysergic acid diethylamide (LSD) decreased brain iron and copper. 2,2′,2″-tripyridine and 3-acetylpyridine also decreased brain iron but their effect on copper was not investigated. Gaidenko (1966) observed a fall in brain iron after injection of niacin. Hanig (1966) and Popov (1967) measured the influence of various drugs on several brain metals, among them iron, copper, and zinc.

There are, however, large discrepancies between the values reported by different authors, especially regarding the iron content. This subject has been reviewed elsewhere (Rafaelsen and Kofod, 1969). As we found essentially lower values for this metal in some preliminary studies, we thought it necessary to reexamine the studies on the influence of various drugs on brain metals. The variation of brain iron, copper, and zinc with age and sex was also investigated.

2. MATERIALS AND METHODS

2.1. *Animals*

For each experiment 20 rats (Wistar strain) were used (food and water *ad libitum*). The animals were 220 g females.

2.2. *Anaesthesia*

The animals were anaesthetized by inhalation of a mixture of 50% CO_2–50% O_2 before the intravenous (i.v.) injections and before sacrifice. No anaesthesia was used before intraperitoneal (i.p.) injections.

2.3. *Dose and administration*

The animals were injected with either LSD (4 mg/kg, i.p.), tremorine dihydrochloride (25 mg/kg, i.p.), 3-acetylpyridine (500 mg/kg, i.p.), or 2,2′,2′-tripyridine (19 μg/kg, i.v.). The control animals were given physiological saline. The i.v. injections were given into a leg vein, the i.p. injections were given to the right of the umbilicus.

2.4. *Procedure*

30 min after the injection, the animal was anaesthetized with CO_2-O_2 and the jugular veins were cut. The abdomen was opened and a hypodermic needle was inserted into the aortic bifurcature into which water was infused until it appeared from the cut jugular veins; 50 ml were sufficient for exsanguination. After decapitation the skull was opened, and the brain removed.

2.5. *Glassware*

All glassware used was cleaned by equal parts of Detex-11 (Produkte für Medizin und Chemie, Solothurn, Switzerland), ethanol and water, followed by rinsing with deionized water.

2.6. *Dry ashing of tissue and determination of iron, copper, and zinc*

The rat brain was dried *in vacuo* at 100°C and ashed in a low temperature asher. The ash was dissolved in 2 ml of 4 N hydrochloric acid, 1.5 ml of 0.25% Sterox SE (Harleco) was added, followed by water up to a final volume of 25 ml. In this solution the concentrations of iron, copper, and zinc were determined by atomic absorption spectroscopy at 2483, 3250, and 2139 Å, respectively; spectral bandwidth was always 3.3 Å.

2.7. *Statistical analysis*

The significance of the difference between the means was calculated using Student's t test; p was obtained from t tables.

3. RESULTS

As iron is an integral part of hemoglobin, contamination with blood is a pitfall when studying iron in tissue. In the present study the mean iron concentration (± S.E.M.) in perfused brains of 220 g rats was 227 ± 5 nmoles per g wet weight. In animals not perfused it was 326 ± 19 nmoles per g wet weight. This difference was significant ($p < 0.001$). The amounts of copper and zinc were independent of blood content.

The perfusion of deionized water instead of a physiological solution might increase the wet weights of the brains due to the entering of water into the cells. However, mean weights (± S.E.M.) for the brains of 220 g perfused and not perfused rats were,

Table 1
Rat brain content of iron, copper, and zinc after injection of sodium chloride, LSD, 3-acetylpyridine, tremorine dihydrochloride, and 2,2′,2″-tripyridine.

	NaCl	LSD (4 mg/kg, i.p.)	3-Acetylpyridine (500 mg/kg, i.p.)	Tremorine (25 mg/kg, i.p.)	2,2′,2″-Tripyridine (19 μg/kg, i.v.)
nmoles/g wet wt.					
Fe	227 ± 5	229 ± 6	231 ± 7	231 ± 6	219 ± 5
Cu	38.9 ± 0.9	38.3 ± 0.8	39.3 ± 0.5	40.6 ± 0.8	38.9 ± 0.7
Zn	198 ± 1	199 ± 3	200 ± 1	199 ± 2	199 ± 2
nmoles/g dry wt.					
Fe	1050 ± 24	1050 ± 25	1070 ± 39	1010 ± 31	1020 ± 24
Cu	179 ± 3	176 ± 4	181 ± 3	171 ± 4	180 ± 3
Zn	914 ± 4	914 ± 12	923 ± 9	872 ± 18	919 ± 7
μmoles/g ash wt.					
Fe	13.0 ± 0.3	13.1 ± 0.3	13.8 ± 0.5	13.5 ± 0.4	12.8 ± 0.3
Cu	2.2 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.1 ± 0.1
Zn	11.3 ± 0.1	11.4 ± 0.1	11.5 ± 0.2	11.7 ± 0.2	11.6 ± 0.1

* Values expressed as mean ± S.E.M. Each group consists of 10 female rats (220 g).

Table 2
Iron, copper, and zinc content in individual rat brains in relation to age and sex.

Age (days)	Weight (g)	Sex	Brain wt. (mg) wet	dry	ash	Dry wt. (%)	Fe	Cu	Zn	Fe	Cu	Zn	Fe	Cu	Zn
							nmoles/g wet wt.			nmoles/g dry wt.			μmoles/g ash wt.		
16	28	M	1300	210	21	16.2	148	27.6	181	917	170	1120	9.3	1.7	11.4
	27	F	1284	237	21	18.4	148	27.9	180	805	151	975	8.9	1.7	10.8
21	51	M	1497	278	25	18.6	163	30.9	194	877	167	1050	10.0	1.9	11.9
	48	F	1448	271	24	18.7	169	31.5	200	904	168	1070	10.3	1.9	12.1
30	105	M	1495	336	27	22.4	172	32.0	199	765	143	887	9.6	1.8	11.1
	104	F	1538	321	27	21.9	199	38.3	198	952	184	950	11.4	2.2	11.3
60	203	M	1551	330	28	21.3	222	43.2	199	1040	203	935	12.2	2.4	11.0
	205	F	1912	428	35	22.4	247	45.2	190	1110	202	848	13.5	2.8	10.4
85	288	M	1806	388	31	21.5	220	35.7	195	1020	166	908	12.8	2.1	11.3
	273	F	1960	440	35	22.4	208	43.9	188	926	196	834	11.5	2.4	10.4

1.79 ± 0.02 and 1.81 ± 0.07 g, respectively. This difference was not statistically significant ($p > 0.5$).

The administration of LSD, tremorine dihydrochloride, 2,2′,2″-tripyridine, or 3-acetylpyridine caused no statistically significant difference in brian iron, copper, or zinc. In table 1 are listed mean values (± S.E.M.) for the three metals, expressed per gram wet weight, per gram dry weight, and per gram ash weight.

The relation of brain iron, copper, and zinc to age and sex is seen in table 2. It was found that iron and copper increased with age, and zinc remained constant, when expressed per gram wet weight and per gram ash weight. Per gram dry weight, iron and copper remained constant, while zinc decreased. There were no differences between males and females regarding iron, copper, or zinc content.

4. DISCUSSION

Iron compounds in tissues may be divided into heme containing and nonheme containing compounds. Under normal conditions hemoglobin is not present in the brain outside the vascular bed, but it appears in the tissue as a consequence of hemorrhage or hemolysis. This hemoglobin is taken up by glial cells and converted to hemosiderin. Other brain proteins containing heme bound iron are the cytochromes and peroxydases, but they probably contribute only to a minor fraction of the total iron (Granick, 1949).

The nonheme compounds belong to the more heterogenous and quantitatively larger group. The major part of iron in this group is generally found in hemosiderin and ferritin. It is also probable that brain tissue contains small amounts of iron in lipid complexes (Tingey, 1938).

As mentioned above, the values obtained for brain iron vary considerably with blood contamination of the tissue. This is a pitfall in analyzing, especially with low total iron content. When studying such tissues, it is necessary either to remove the blood completely or to use methods by which hemoglobin iron is not determined (Hallgren and Sourander, 1958).

In view of this, the results of Hadžović, Nikolin, and Stern (1965, 1967) and of Sowell (1967) are of special interest. The former authors perfused the brains in a similar way as applied in the present study. They nevertheless found values sevenfold the present ones for iron (table 3). On the other hand, Sowell (1967), who did not take any precautions for excluding hemoglobin iron, found half as much iron in the brain. The discrepancies may be explained by variations in procedures particularly since incineration without taking necessary precautions will lead to erroneous values.

The values for iron are lower than those found by most other authors, but it should be noted that values

Table 3
Different authors' findings for iron, copper, and zinc in brains.
The values are metal concentrations in nmoles per g wet weight.

Species	Iron	Copper	Zinc	Reference
Rat	1580	–	–	Hadžović, Nikolin and Stern (1965)
	–	35	–	Hadžović, Košak and Stern (1966)
	128	34	132	Sowell (1967)
	227	39	198	Kofod (1970), present results
Rabbit	181–444	41–86	165–243	Hanig (1966), Hanig and Aprison (1967)
	730	43	158	Voroskilovskaya and Raitzes (1966)
	–	36	172	Popov (1967)
Calf	–	11–63	62–310	Wong and Fritze (1969)
Cow	–	8–37	68–303	Wong and Fritze (1969)
Pig	–	23–73	55–234	Wong and Fritze (1969)
Cat	1080	–	–	Gaidenko (1966)
Dog	130–242	–	–	Duschechkina (1949)
Human	72–265	33–76	–	Tompsett (1935)
	680–3940	–	–	Tingey (1938)
	771	70	157	Griffith, Butt and Walker (1954)
	610	94	–	Dorfman and Schipitsyn (1955)
	180–3800	–	–	Hallgren and Sourander (1958)
	134–3180	36–362	101–2990	Löwenthal (1959)
	680–1500	66–139	139–296	Perry et al. (1962)
	95–2580	67–252	–	Musil et al. (1964)
	–	–	130–235	Chang, Gover and Harrison (1966)
	452–2200	35–156	138–306	Harrison, Netsky and Brown (1968)
	466–2940	43–143	92–336	Backer (1969)

* When two values are listed, they are, respectively, the lowest concentration (generally in white matter) and the highest (generally in some of the basal ganglia).

close to our rat data were obtained by Tompsett (1935) in humans, and by Duschechkina (1949) in dogs.

The values for copper and zinc in the present work are of the same order of magnitude as the results obtained by other authors (table 3).

Our pharmacological studies were performed mainly to repeat the experiments of Hadžović and coworkers (1965, 1966, 1967). In their study 2,2′,2″-tripyridine, 250 μg/kg, administered to rats decreased brain iron. We found it impossible to use the same dose since 25 μg/kg was lethal. After about 20 μg/kg, the animals developed intense tremors, but none died within the next 30 min. However, even with this dose no decrease in brain iron occurred.

Hadžović and coworkers (1965, 1966, 1967) also reported that injection of LSD, tremorine, and 3-acetylpyridine to rats caused statistically significant decreases in brain iron and copper. The present work does not confirm these findings (table 1).

Coggeshall and MacLean (1958) injected 3-acetylpyridine in mice in a dose of about 300 mg/kg body weight. Four hours after injection, they observed a destruction of the cells in the same areas of the hippocampus which Maske (1955) reported to be specifically stainable for zinc *in vivo* after injection of dithizone. In the present work, where higher doses (500 mg/kg) were applied, no changes in rat brain zinc concentration were observed after 30 min. It should, however, be of interest to see whether there is a correlation between brain zinc content and the degree of cell destruction in the hippocampus.

Rat brain iron and copper increased with age (table 2). This corresponds to similar findings in human brain for iron, reported by Hallgren and Sourander (1958) and by Sundermann and Kempf (1961).

The presence of 'stainable' iron in brain was demonstrated by Guizzetti (1915) and Spatz (1922) by treating thick, macroscopic brain sections with potassium ferrocyanide or ammonium sulfide. In some areas of the brain a colour then developed, due to the presence of iron in the tissue. This stainable iron does not contain hemosiderin, carbohydrates, or lipids, but at least a fraction of it seems to be identical to ferritin (Diezel, 1954).

The finding of an increase in rat brain iron with age is also in agreement with the distribution of stainable iron by Guizzetti (1915) and Spatz (1922). In young humans they found that only the globus pallidus showed a positive iron reaction and that with increasing age other nuclei also showed the reaction, in the following order: substantia nigra, nucl. ruber, nucl. dentatus, nucl. caudatus, putamen.

Similar to this increase in stainable brain iron accompanying growth in man, an increase in stainable brain iron has been observed in the animal kingdom according to the evolution of species. Guizzetti (1915) and Spatz (1922) found the following sequence with respect to the intensity of the iron reaction: Mouse, guinea pig, rabbit, cat, dog, rhesus monkey, and a maximal reaction in man.

According to Guizetti (1915) and Spatz (1922) the brains of mouse and guinea pig contain virtually no stainable iron, and only very small amounts are found in rabbit brain. The rat was not examined by these workers, but as it is evolutionary close to the mouse, it is likely that rat brain contains none or only very small amounts of stainable iron. Contrary to these lower animals, the brains from dog, monkey, and man showed very distinct iron reactions. Mouse brain contains about the same concentration of total iron as rat brain (Kofod, to be published); Hanig (1966), Duschechkina (1949), and Tompsett (1935) also found values close to our rat data for rabbit, dog, and man respectively (table 3).

Thus, the brains from animals in varying evolutionary state contain equal amounts of brain iron, but varying amounts of stainable iron. From this one may assume that stainable iron, which is believed to consist partly of ferritin (Diezel, 1954), only constitutes a minor fraction of total iron in brain, and also that it is only this fraction that varies with age and evolutionary state of the animal, while the major fraction of brain iron remains constant.

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