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QUANTITATIVE EVALUATION OF TRANSPORT OF SOME LABELLED COMPOUNDS INTO RAT CEREBRAL TISSUE UNDER NORMAL CONDITIONS AND DURING ISCHAEMIA AND HYPOXIA OF BRAIN

A. KAPUSCIŃSKI, M. J. MOSSAKOWSKI
Department of Neuropathology, Medical Research Center, Polish Academy of Sciences, Warsaw, Poland

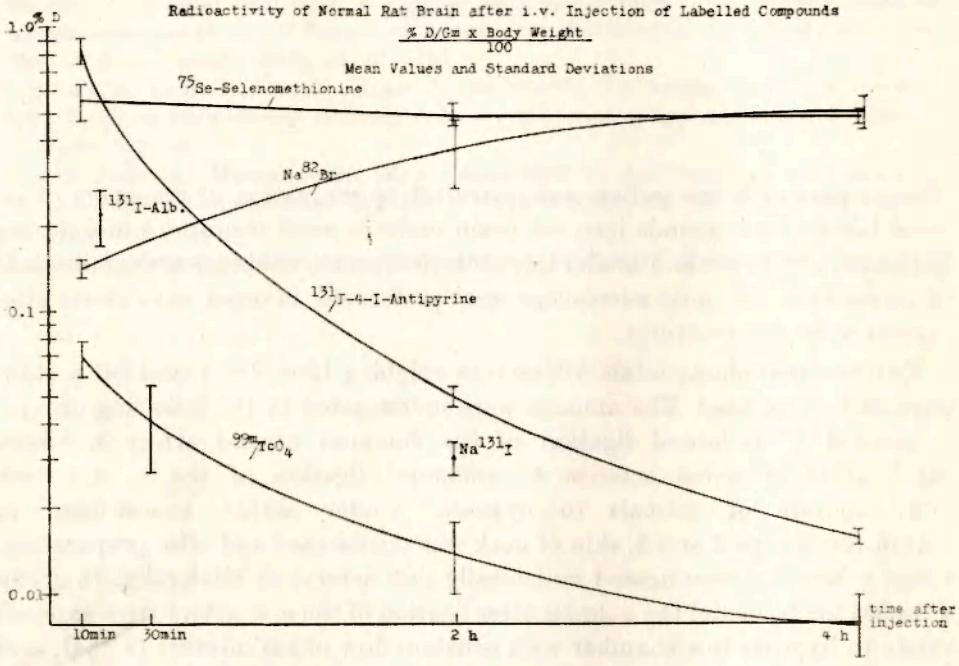
The purpose of investigation was quantitative evaluation of transport of several labelled compounds into rat brain under normal conditions and during ischaemia and hypoxia. Parallel morphological and histochemical examinations of glycogen in the optic microscope were performed, in order to evaluate alterations in brain structures.

Two hundred ninety adult Albino rats weighing 150—250 g. and fed a standard diet, were used. The animals were investigated in the following groups: 1. control 2. unilateral ligation of the common carotid artery 3. bilateral ligation of these arteries 4. unilateral ligation of the c. c. artery and exposure of animals to hypoxia. Under aether anaesthesia, in rats of the groups 2 and 3, skin of neck was transsected and after preparation, the c. c. arteries were ligated unilaterally (left artery) or bilaterally. In group 4 (ischeamic-hypoxic) the animals after ligation of the c. c. artery were exposed singly to hypoxia in a chamber with constant flow of gas mixture (4 % O_2 and 96 % N_2). The composition of the gas mixture was determined volumetrically with control of the partial oxygen pressure by use of Clark's electrode. When apnoea occured, the animals were removed from the chamber until spontaneus respiration returned. In a few cases they were artificially ventilated. Thereafter the animals were again exposed to hypoxia in chamber, within a standard period of 30min.

The following labelled compounds were used: $^{99m}TcO_4$, ^{75}Se-selenomethionine, ^{131}I-4-I-antipyrine, $Na^{131}I$, $Na^{82}Br$ and ^{131}I-albumin. After ligation of the c. c. arteries, the compounds were injected into the femoral vein in doses of 4—40 μCi and volume 0.1—0.7 ml. The animals were killed after survivals of standard periods of 10 min to 5 h and in some case 96 h after inducing of pathology. For radioactivity measurements 1 ml blood and the brain with cerebellum was taken. The brain hemispheres were cut apart along the corpus

callosum and weighed. The activity of each hemisphere and of the blood sample was measured in the well-type scintillation counter. The results were expressed for each hemisphere as % D/g corrected for body weight and as a ratio of brain to blood specific activity. The ratio of specific activity of the left and right hemisphere as well a the % D in whole blood were also calculated. In one group of animals the whole blood volume and the brain blood volume were determined by use of ^{131}I-albumin.

Figure 1.
Radioactivity of Normal Rat Brain after i.v. Injection of Labelled Compounds
$\frac{\% \text{ D/Gm x Body Weight}}{100}$
Mean Values and Standard Deviations



The radioactivity of normal rat brain after i. v. injection of labelled compounds as a function of time is shown in fig. 1.

Antipyrine penetrates rapidly into the brain reaching a high concentration and quickly disappears from the brain. ^{75}Se-selenomethionine penetrates also rapidly into the brain (its concentration is, however, lover as compared with that of antipyrine) and the activity of brain remains approximately at the same level in later periods. Penetration of ^{82}Br into the brain is much slower and its concentration rises up to 4 h after injection. Only minimum quantities of $^{99m}TcO_4$ reach the brain, so that the concentration of this compound is low and depends mainly on the radioactivity of blood. The activity of brain 2 h after injection of Na ^{131}I is about twice higher as compared with that after $^{99m}TcO_4$. The brain activity after injection of ^{131}I-albumin is relatively high,

because of its high concentration in blood as compared with that after injection of other compounds. The ratios of brain/blood specific activities 10 min after injection of compounds were as follows: ^{75}Se-selenomethionine 78.8 %, ^{131}I-4-I-antipyrine 51.0 %, Na ^{82}Br 5.4 %, $^{99m}TcO_2$ 2.3 % and ^{131}I-albumin 2.0 %.

The concentration of labelled compounds in the rat brain after unilateral and bilateral ligation of the c. c. artery is presented in fig. 2.

There were no statistically significant differences as compared with the control groups in the concentration of these compounds after unilateral and bilateral ligation of the c. c. artery, with two exceptions for the bilateral ligation group; 4 h after ligation and injection of $^{99m}TcO_4$ as well as 24 h after ligation and 2 h after injection of 75Se-selenomethionine, the concentration of these compounds were slightly higher. This might have been due to an error connected with the injection of higher dose, since the brain/blood ratio in these sub-groups did not differ statistically significant as compared with the control groups.

The experiments seem to indicate that unilateral and bilateral ligation of the c. c. artery in rats does not essentially influence the blood supply of brain. Morphological and histochemical examinations did not show significant changes in the brain structures, except slight glycogen deposits appearing 2 h after bilateral ligation.

In the ischaemic-hypoxic group, transport of individual compounds to the brain as well as the brain structures shows significant changes. As an example of these changes, the results of experiments by use of $^{99m}TcO_4$ are presented in fig. 3.

In most cases, within the first two hours after exposure of animals to low oxygen conditions, both the measured and the specific activity of the left hemisphere were lower as compared with the activity of the right one. In later periods in the majority of cases activity of the left hemisphere was much higher as compared with that of right hemisphere and activity of brain in control groups. The weight ratio of both hemispheres shows the appearance of oedema of the left hemisphere as early as in the first hour of the experiments.

The results indicate a change of permeability of the vessels between the 1st and 2nd hour after brain ischaemia and hypoxia leading to an intensive penetration of $^{99m}TcO_4$ into the injured hemisphere. Morphological examinations confirmed the appearance and development of brain oedema and progressive lesions of its structures.

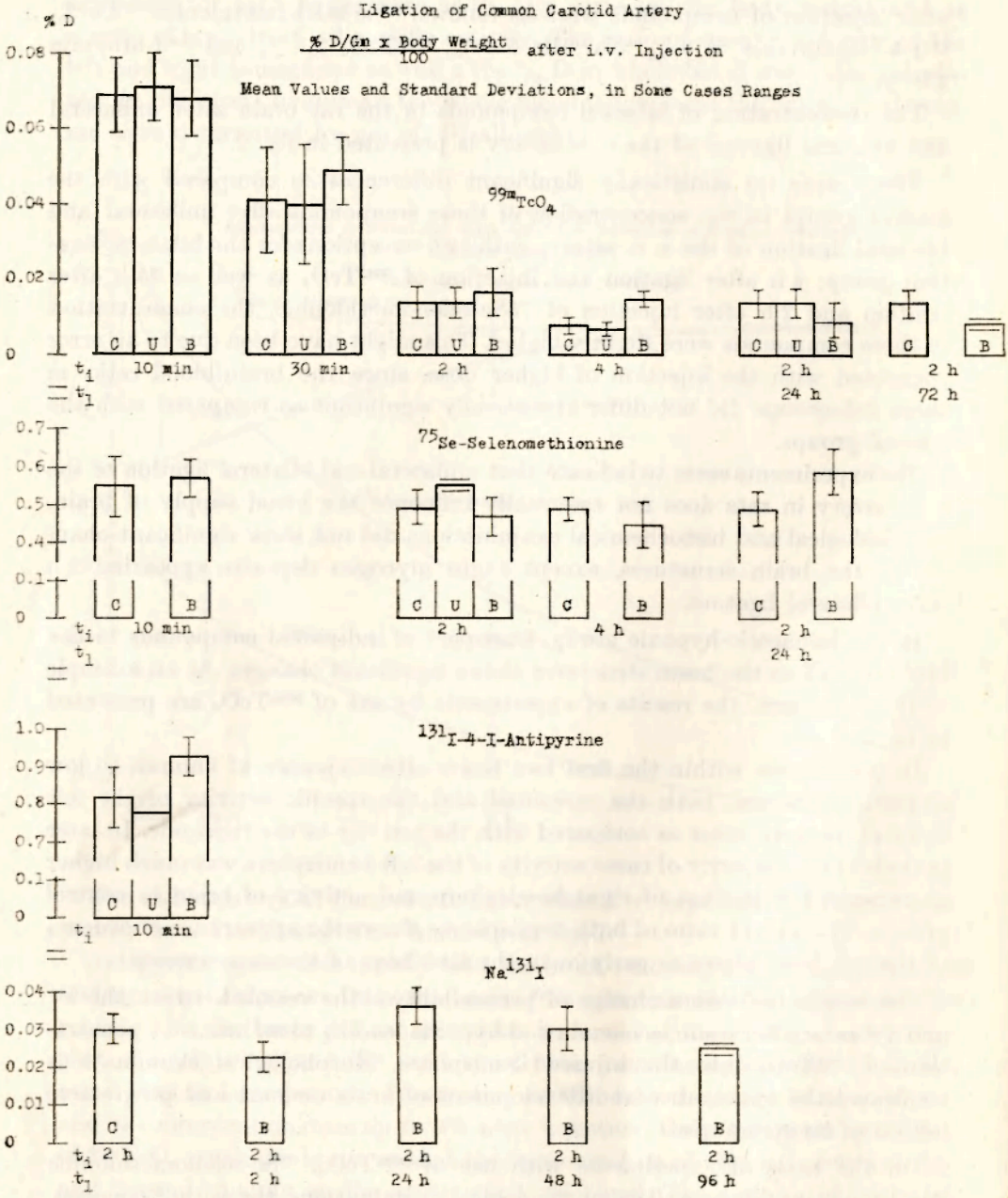
On the basis of experiments with use of $^{99m}TcO_4$, ^{75}Se-selenomethionine and ^{131}I-antipyrine as well as morphological examinations, the authors suggest, that the ischaemic factor in this model is mainly responsible for the development of oedema with progressive damage of brain.

Figure 2.

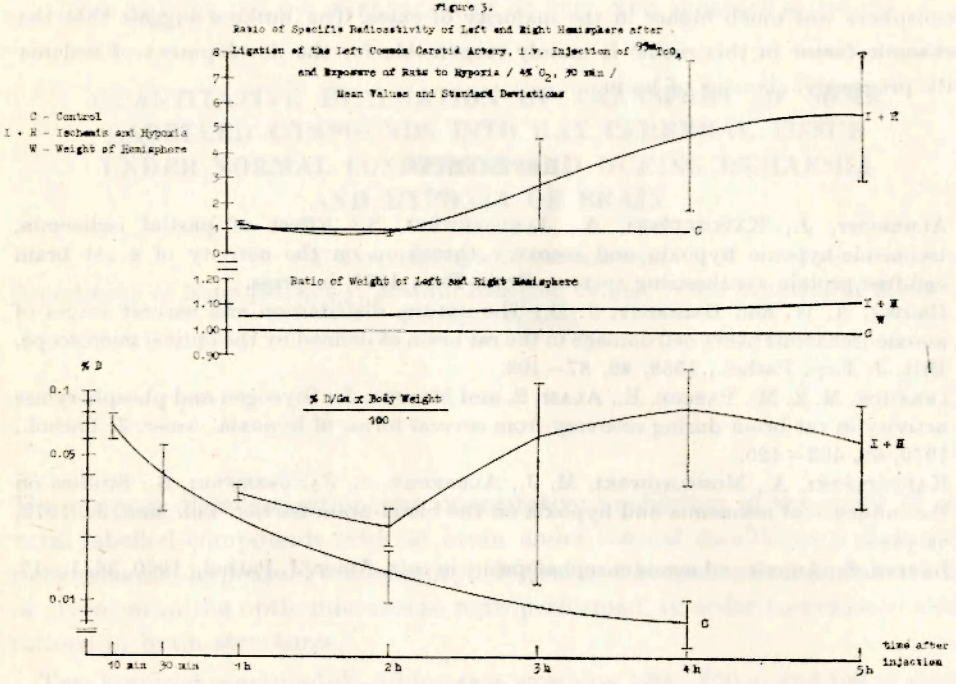
Concentration of Labelled Compounds in Rat Brain after Unilateral and Bilateral Ligation of Common Carotid Artery

% D/Gm x Body Weight / 100 after i.v. Injection

Mean Values and Standard Deviations, in Some Cases Ranges



C - control
U - unilateral ligation
B - bilateral ligation
t_i - time after injection of compounds
t_1 - time after ligation



SUMMARY

Adult Albino rats (290) were investigated under aether anaesthesia in the following groups: 1. control 2. unilateral ligation of the common carotid artery 3. bilateral ligation of these arteries 4. unilateral ligation of the c. c. artery and exposure to anoxia or hypoxia (4 % O_2). I—HSA, $vTcO_4$, Na I, Na Br, I—4—I—antipyrine and Se — selenomethionine were injected i. v. in all of these groups. Parallel optic microscopic and histochemical (glycogen) studies vere performed. The activity of each hemisphere and of a blood sample were measured in a well — type scintillation counter mainly between 10 min and 5 h and in some cases up to 96 h after induction of pathology. The results were expressed for each hemisphere as: *M* D/g corrected for body weight, ratio of brain/blood specific activities and *M* D in whole blood. In one group of animals, whole blood volume and the brain blood volume were determined. Comparative transport into normal cerebral tissue was as follows: ^{131}I—4—I—antipyrina ^{75}Se—selenomethionine Na^{82}Br Na^{131}J $^{99m}TcO_4$ ^{131}J —HSA. There were no statistical differences as compared with the control group in the transport of these compounds into brain tissue after unilateral and bilateral ligation of the c. c. artery. Anoxia (5 min) and hypoxia up to 3 h caused no changes in transport of these compounds. In the ischaemic—hypoxic group penetration of these compounds into the damaged

hemisphere was much higher in the majority of cases. The authors suggest that the ischaemic factor in this model is mainly responsible for the development of oedema with progressive damage of brain.

REFERENCES

1. Albrecht, J., Kapuściński, A., Januszewski, S.: Effect of partial ischaemia, ischaemic-hypoxic hypoxia and recovery therefrom on the activity of a rat brain cell-free protein synthesizing system. Brain Res., 1971, in press.
2. Brown, A. W. and Brierley, J. B.: The nature, distribution and earliest stages of anoxic-ischaemic nerve cell damage in the rat brain as defined by the optical microscope. Brit. J. Exp. Pathol., 1968, 49, 87—106.
3. Ibrahim, M. Z. M., Pascoe, E., Alam, S. and Miquel, J.: Glycogen and phosphorylase activity in rat brain during recovery from several forms of hypoxia. Amer. J. Pathol., 1970, 60, 403—420.
4. Kapuściński, A., Mossakowski, M. J., Albrecht, J., Januszewski, S.: Studies on the influence of ischaemia and hypoxia on the blood-brain barrier. Pol. Med. J., 1972, in press.
5. Levine, S.: Anoxic-ischaemic encephalopathy in rats. Amer. J. Pathol., 1960, 36, 1—17.