DISTRIBUTION OF RADIOACTIVE SODIUM AND CHANGES IN INTRAOCULAR TENSION DURING CARBONIC ANHYDRASE INHIBITION

(UDC 617.7-007.681-085.363 : 577.154.4]-073.916)

S. M. Mintz, G. M. Butenko, and A. G. Reznikov

Department of Pathological Physiology, Odessa Medical Institute
(Presented by Active Member AMN SSSR A. V. Lebedinskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 58, No. 11, pp. 42-45, November, 1964
Original article submitted July 18, 1963

The inequality of the distribution of substances in the organism and the preservation of a constant composition both in the entire organism and in its different parts is related to the functions of biological membranes (the renal tubules, ciliary epithelium of the eye, the choroid plexus of the brain, the entire mechanism of the blood-brain barrier, etc). Many substances are actively transported and, therefore, their distribution is closely related to cellular enzymatic processes. It has recently been shown [2, 3, 6] that the enzyme carbonic anhydrase participates in the process of active transport. For example, it has been discovered [4] that carbonic anhydrase plays a major role in the formation of aqueous humor affecting the secretory activity of the ciliary epithelium. For this reason it was suggested that carbonic anhydrase inhibitors be used in the treatment of glaucoma [4]. Inhibitors of this enzyme have found wide application in clinical practice for the treatment of a number of other pathological processes (diuretic and anti-convulsive actions). Acetazolamide (diacarb, fonurit, diamox) is the most active of these inhibitors.

This investigation aimed at studying the effect of acetazolamide on the distribution of Na^{24} in the organism and the dynamics of changes in intraocular tension.

METHOD

The experiments were carried out on white rats (a total of 60 experiments). Fonurit (Hungarian preparation, synonym of acetazolamide) was given subcutaneously as a 5% solution (100 mg/kg). After 5 min post injection, Na^{24} in a dose of 50 microcuries/kg was given intravenously in a volume of physiological solution equal to 0.25 ml per 100 gm live weight. The rats were sacrificed by exsanguination at 15, 30, 60 and 120 min after administration of the isotope. The radioactivity of the renal tubules, blood serum, and slices of brain, kidney and liver was determined on a B-2 apparatus with an AC-2 counting tube, placed in a lead housing. The results were calculated as relative activities (the radioactivity of the blood serum was taken as 100%).

Intraocular tension in all animals was measured with a Maklakov type tonometer (weight 2 gm) before administration of the fonurit and before the death of the animals. In ten animals only the changes in intraocular tension were measured during the 2 h after fonurit administration.

The means of results obtained in different series of experiments were compared. Verification of the differences of the means was determined statistically.

RESULTS

We were first interested in the distribution of Na^{24} in control animals which did not receive fonurit.

The results of this series of experiments showed that during the first 5 min after intravenous administration of Na^{24} the serum radioactivity fell rapidly. This may be explained as the rapid establishment of isotopic equilibrium between the blood serum and the so-called sodium space of the internal organs. After 5 min the curve of blood radioactivity began to show a rather flat slope related to the slower operation of sodium metabolism processes. The radioactivity of the internal organs, for example, the liver, sharply rose over the first 5 min and then reached a constant level.

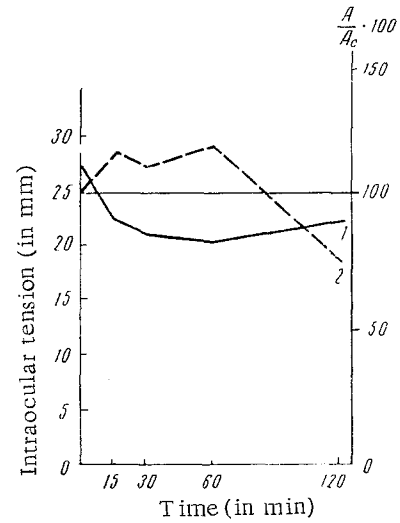

Fig. 1. Interrelationship between changes in intraocular tension and Na^{24} penetration into the chamber fluid after administration of fonurit in a dose of 100 mg/kg. 1) Intraocular tension; 2) per cent ratio of chamberfluid radioactivity in the experiment to radioactivity in the control A/A · 100. Abscissa: time (in min). Ordinate: interocular tension (in mm).

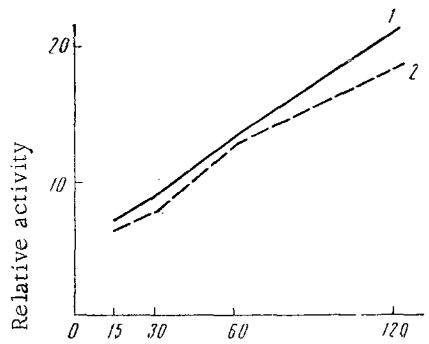

Fig. 2. Na^{24} penetration into tissues of rat brain in the normal (1) and after administration of fonurit in a dose of 100 mg/kg. Abscissa: time (in min). Ordinate: relative activity.

The penetration of Na^{24} into the brain and the anterior chamber of the eye takes place extremely slowly. Isotopic equilibrium between blood serum and the humor of the anterior chamber is established only at the end of the second hour, and in the brain has not even begun at that time.

In a dose of 100 mg/kg, fonurit evokes a number of changes in the Na^{24} distribution in the organism. These changes concern first the penetration of Na^{24} in the eye, kidneys and brain. In the anterior chamber the aqueous humor content of the isotope during the first hour is considerably higher than in the control, but later, on the contrary, it falls below normal levels (Fig. 1).

It is interesting to note that changes in the intraocular tension after fonurit administration are contrary; that is, with increase in the Na^{24} concentration in the anterior chamber of the eye, the intraocular tension decreases and, on the contrary, a decrease in the isotope content in the aqueous humor corresponds with initiation of normalization of the intraocular tension.

Evidently, an increase in Na^{24} concentration in the chamber fluid over the first hour after administration of acetazolamide is related to the decrease in water transport.

In experiments on rabbits, various inhibitors of carbonic anhydrase (diamox, dibenemid and many diuretic dichlorphenamides) did not affect the sodium level in the chamber fluid while they lowered the intraocular tension [5].

The observed divergence between Na^{24} concentration in the chamber fluid and the level of intraocular tension confirms the validity of doubts [2] expressed about the concept that hypertonicity of the chamber fluid ensures the transport of water into it and affects the intraocular tension. These doubts have led to a hypothesis concerning pinocytosis and the role of this process in secretion. The question is that of the movement of water and water-soluble substances across the epithelial cells of the ciliary body [7]. By pinocytosis is meant the capture of droplets of liquid medium containing dissolved substances by the surface cells in a manner analogous to phagocytosis of solid particles [1]. It is suggested that the pinocytosis phenomenon, as one of the widespread mechanisms for the majority of animal cells, may also be important in active transport and the creation of ionic pumps [3, 8].

Finally, electron microscopic studies give direct evidence for an inhibitory affect on pinocytosis from diamox. Thus, Holmberg [6] noted an increase in the number of bubbles in the cytoplasm of the ciliary epithelium within 15-30 min after intravenous administration of diamox. This observation corresponded temporally with the delay in aqueous humor secretion. Later, the number of bubbles decreased to normal.

In our experiments at the end of 2 h after fonurit and isotope administration the relationships had changed; intraocular tension began to return gradually toward normal, i.e., to increase while the Na^{24} concentration fell in comparison with the control (see Fig. 1). This but again is a function of water transport: the fonurit effect weakens and together with this the entrance of water from the ciliar epithelial cells into the ocular chamber is resumed.

It is of further interest to study the effect of fonurit on the transmission of Na^{24} in the kidney. As our observations have shown, the Na^{24} concentration in kidney tissue undergoes similar changes to the Na^{24} concentration in the aqueous humor: initially the radioactivity increases and subsequently falls in comparison with normal; however, the instantaneous exchange does not decrease with time.

The similarity in radioactive changes in the kidney and the eye once again testifies to the generality of the physiological process of secretory action of the ciliary epithelium and the kidney tubules and to the role of carbonic anhydrase in this process. This functional similarity is confirmed by the morphological data. Electron microscopic studies have permitted the detection of the same time of beta-cytomembranes in cells of the ciliary epithelium and of the kidney tubules. These membranes evidently play some role in the exchange of ions between cells.

Our experiments also show a delayed penetration of Na^{24} in the brain, as compared with controls. This appears during the 2 h observation period (Fig. 2).

As far as the isotope concentration in the serum, this differs only slightly from normal. At the same time the Na^{24} concentration in liver increases significantly as compared with the control (from 19.2 ± 0.3 to $22.4 \pm 0.6\%$) which indicates an increase in the hepatic "sodium space."

LITERATURE CITED

1. A. V. Zelenin, Uspekhi sovr. biol., 3, 53 (1962), p. 364.
2. B. Becker, Am. J. Ophthal., 47 (1), Pt. 2 (1959), p. 342.
3. H. S. Bennet, J. biophys. biochem., 2, Suppl., Cytol (1956), p. 99.
4. J. S. Friedenwald, Am. J. Ophthal., 32, Pt. 2 (1949), p. 9.
5. J. E. Harris, A. E. Carlson, L. Gruber, et al., Ibid., 44, N 5, Pt. 2 (1957), p. 409.
6. A. Holmberg, Arch. Ophthal., 62 (1959), p. 935.
7. S. Lerman, Glaucoma; Chemistry, Mechanisms and Therapy. Springfield (1961).
8. G. E. Palade, J. biophys. biochem., 2, Supp., Cytol. (1956), p. 85.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. ***Some or all of this periodical literature may well be available in English translation.*** **A complete list of the cover-to-cover English translations appears at the back of this issue.**