ON THE EFFECT OF CERTAIN ANESTHETIC AGENTS ON THE STRUCTURAL TEMPERATURE OF ISOLATED LIVING TISSUES

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If in ordinary water the translational motion of its molecules is determined by the intrinsic structure of the water, by the height of the potential barrier between neighboring positions of equilibrium of the molecules and temperature, then the entire complex ensemble of the physicochemical situation and the architectonics of the living cell have an effect on self-diffusion of the molecules of the tissue water. Therefore, any change of the colloid-chemical state of the protoplasm should be accompanied by a change in the self-diffusion coefficient of tissue water and consequently of the structural temperature of tissues also.

Appreciable colloid-chemical changes in tissues under anesthesia have been noted by authors [1] who used various method of investigation.

In the present work we attempted to characterize the colloid-chemical changes in isolated living tissues under the effect of certain anesthetic agents by recording the changes of the so-called structural temperature of tissue.

The structural temperature was determined by direct correlation of the values of the self-diffusion coefficient of tissue water found empirically and the tabular data of the temperature dependence of this coefficient for pure water [6, 8].

In the work we used the nuclear spin-echo technique which yielded direct information on the coefficient of self-diffusion of tissue water which characterizes the hydration of the structural and functional components in the living cell.

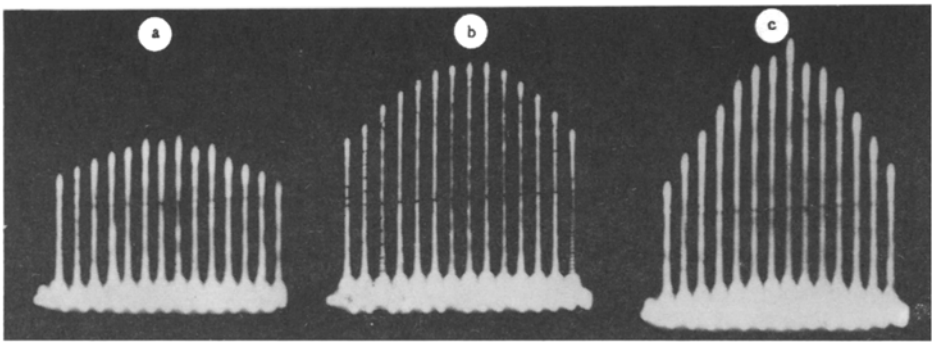

Fig. 1. Change of the envelope of echo signals under the effect of self-diffusion in tissue of liver (a), muscular tissue (b), and pure water (c).

EXPERIMENTAL METHOD

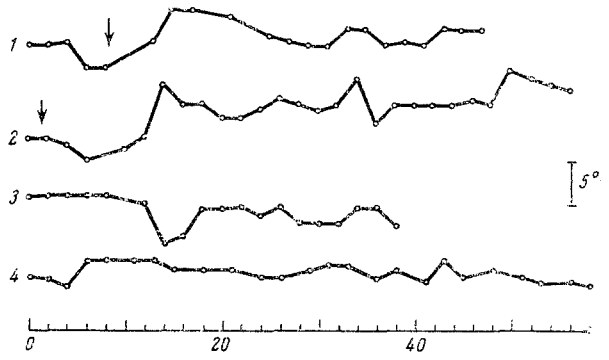

Fig. 2. Effect of certain volatile anesthetics on the structural temperature of the gastrocnemius muscle of frogs. 1) Ether vapors; 2) chloroform vapors; 3) postanesthetic changes after 10 min exposure in Ringer's solution saturated with diethyl ether; 4) intact muscle. At the bottom are the time markers (in min) and on the right side, the scale of the changes in the structural temperature.

The experiments were carried out on isolated tissues of Rana esculenta (15 frogs). After extraction the tissues were placed in a thin-walled graduated measuring glass tube with a maximal achievable space factor without disturbing anatomical integrity. To ensure a cylindrical "cut" of tissue we used brass electrostatic cylindrical "screens" which were inserted from both sides into the measuring tube until they contacted the edge of the tissue sample, which nicely filled the lumen of the tube. The anesthetic agent was used as a vapor when the reservoir containing the particular volatile anesthetic, with a limited 3 mm diameter surface of evaporation, was connected to the lower end of the measuring tube. Preliminary observations of the gastrocnemius muscle showed that this action of the anesthetic is effective and regularly leads to the development of an evident reversible contracture which usually occurs under isometric conditions. In other cases an anesthesized state was achieved by immersing the investigated tissue for 10 min into Ringer's solution saturated with diethyl ether and we investigated the postanesthetic state of this tissue.

Measurements were carried out on a specially designed nuclear spin-echo device built around a permanent magnet at a frequency of 19.3 Mc. The amplitude of the pulsed radio frequency field was on the order of 15 oersted. The gradient of the field was from 1 to 1.5 oersted. The gradient was estimated by the width of the echo signal between 2 evident minima. Measurement of the transverse and longitudinal relaxation times (T_2 and T_1) was done according to the methods of Carr-Purcell [3] and Hahn [5].

The self-diffusion coefficient of water was measured by the method of Ghosh and Sinha [4]. The results were photographically recorded from the screen of the oscilloscope. Measurements of the curve of the echo signals under the effect of self-diffusion were carried out to compare the envelope (Fig. 1). All measurements were carried out at 26-30°. The results were processed by the methods cited in the works of the aforementioned authors [3-5].

EXPERIMENTAL RESULTS

Effect of Certain Volatile Anesthetics on the Gastrocnemius Muscle of Frog

Figure 2 shows the result of 4 experiments on the study of the effect of volatile anesthetics on striated muscle. The curves of the dependence of the self-diffusion coefficient of tissue water. The values of the points characterizing intact muscular tissue reflect the structural temperature of striated muscle (the structural temperature of the muscle was 10-15°, which corresponds to a self-diffusion coefficient of tissue water of $1.4\text{-}1.8 \cdot 10^{-5}$ cm/sec). On the right in Fig. 2 is the scale which gives an idea about the relationship between the fluctuations of the self-diffusion coefficient and the changes of the structural temperature in this recording limit.

The effect of both anesthetics led to a rise of the structural temperature by at least 5°. The reversibility of the anesthetic state of the tissue is indicated by curve 3 which pertains to a muscle in which the self-diffusion coefficient was measured after 10 min exposure in Ringer's solution saturated with ether. This curve is characterized by a great constancy of the arrangement of significant points, however, after 10 min it markedly dropped, reflecting the drop of structural temperature by more than 5°. Then the curve somewhat rises against a background of lowered temperature as compared with the anesthetic level, so that by the 40th min it again returns to a value close to the structural temperature of the intact muscular tissue. The fluctuations of the structural temperature of the intact muscle were mainly associated with fluctuations of the functional state of the muscular tissue itself which apparently arose under the effect of unaccounted for stimuli: the appearance of this effect was also due to apparatus instability which generally was of less significance (compare Fig. 3, curve 4).

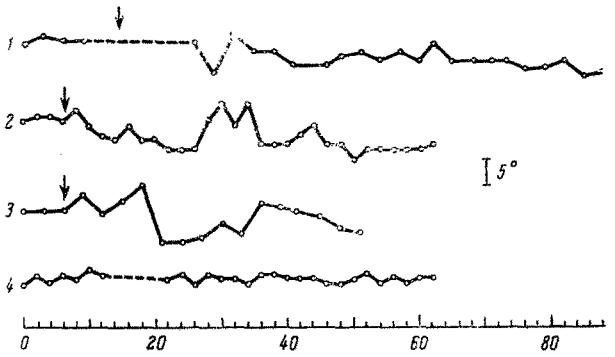

Fig. 3. Effect of volatile anesthetics on the structural temperature of hepatic tissue. 1) Ether vapors; 2) vaporous chloroform; 3) mixture of vaporous ether and chloroform (2:1); 4) intact hepatic tissue. Other designations are the same as in Fig. 2.

Effect of Certain Volatile Anesthetics on the Structural Temperature of Frog Liver Tissue

Figure 3 illustrates the character of the change in the structural temperature of liver tissue under the effect of the vapors of ether, chloroform, and their mixtures. The principle of plotting the curves is the same as that in Fig. 2, however, the value of the structural temperature of the liver is near zero, which corresponds to a self-diffusion coefficient of about $1 \cdot 10^{-5}$ cm^2/sec. The effect of both anesthetic agents and their mixtures caused an appreciable drop in structural temperature of liver tissues. The effect of ether was less evident, drawn out in time, and characterized by a comparatively smooth drop of the structural temperature during the entire course of measurements until a drop of 5° was reached.

Chloroform acted more abruptly, causing a rapid, deep drop of the structural temperature with an episodic rise in the middle of the measurement against a general background of a low structural temperature. A "chloroform" type of change with sharp fluctuations of the shape of the curve appeared sooner in the effect of a mixture of chloroform and ether.

Thus, the action of anesthetic agents causes in the tissues of liver and muscle a substantial change of the translational motion of water, which characterizes the changes of its structure. Different tissues (liver, muscle) yield opposite reactions under the effect of the same anesthetic, although approximately the same types of reactions of different tissues are also possible; thus, in our experiments the hepatic tissue reacted in the same way as the skin tissue of the frog to one and the same anesthetic. Possibly we are dealing with a type of reaction which is characteristic for any group of tissues.

As regards certain physicochemical systems, it was demonstrated that ether can lower the structural temperature of a solution by 30° [2] due to pronounced hampering of the translational motion of water molecules by filling the cavities of the water structure with molecules of this nonelectrolyte. If we assume together with certain authors that the intracellular water is free water, then the results of our experiments on the effect of ether on the translational motion of water molecules in pure water seemingly contradict this, since according to our data ether does not have a noticeable effect on translational motion of molecules in pure water.

On the other hand, the intracellular (tissue) water does not have all the evident properties of pure free water. Thus, for tissue water of muscles T_2 and T_1 differ by more than a factor of 10 (T_2 = 45-60 msec, and T_1 = 700-850 msec), whereas in an ideal fluid and even in free water they should be approximately the same. This could characterize any crystal hydrate properties of tissue water resembling ice if there was no relatively large value of the self-diffusion coefficient. Apparently we are encountering here not only the special structure of water, but also the appreciable orderliness of the interaction of cellular structures with it, which is indicated by the brief time of the spin-spin relaxation (T_2). Therefore, we cannot exclude the mechanism which was proposed [2] for explaining the behavior of special structures of water in certain systems under the effect of an anesthetic, since it is not known to what degree the structure of the intercellular water corresponds to the structure of models investigated by authors, although the opposite nature of the reaction of different tissues (muscle, liver) in our experiments evidently requires a different explanation.

We do not have at our disposal sufficient facts to discuss the other physical theory of anesthesia proposed recently by Pauling [7]. According to this author different polar groups can form around themselves crystal hydrates of a clathrate type at temperatures below the body temperature of warm-blooded animals. However, under the effect of anesthetics there can be tendency toward the formation of mixed crystal hydrates whose stability is appreciably higher and which can already exist at the body temperature of an animal. The crystal hydrates formed can disturb intracellular communication. This point of view the author substantiates by plotting parallel series of the intensity of the anesthetic effect and of crystal hydrate-forming properties of a number of anesthetic agents. We assume that the mechanisms proposed by the aforementioned researchers [2, 7] can be the initiative primary mechanisms of a physical

character which cause secondary profound reorganizations of the functional structures of the cell and cellular water, which include the specific reaction we noted to the effect of anesthetic agents.

Thus, it was shown in the study that the characteristic parameters of the proton system of tissue water (the time of longitudinal and transverse relaxation T_1 and T_2, as well as the self-diffusion coefficient) substantially differ from those in free water. It was also demonstrated that the effect of anesthetic agents leads to a change in the structural temperature of tissue. The character of the changes of this index under the effect of anesthetic agents depends on the type of tissue.

SUMMARY

Direct measurements of self-diffusion coefficients of water in the living tissues isolated from Rana esculenta were made by nuclear spin-echo technique.

The structural tissue temperature was estimated by comparing the experimental values of self-diffusion coefficients of tissue water and the standard data of coefficients for pure water, depending on the temperature.

Anesthetics (ether, chloroform) increased the structural temperature of the skeletal muscle and decreased that of the liver. In both cases the oscillation amplitude of the structural temperature is more than 5°C.

The spin-lattice relaxation time (T_1) and the spin-spin relaxation time (T_2) differed by more than 10 times in the skeletal muscle. The difference reflects the peculiarities of the tissue water (as compared to the free pure water) and characterizes the high degree of organization of the tissue water effected by the structural components of the living cell.

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