These results thus indicate that intravenous injection of PI promotes its rapid absorption in the heart of experimental animals (rabbits), and that luminescence of PI on ultraviolet excitation (λ = 266 nm) is observed mainly in the septal region of the right atrium. This encourages the hope that PI will be used as LS in the future for luminescence probing of the heart as an aid to the solution of medical diagnostic problems.

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REVERSIBLE FUNCTIONAL BLOCKING OF THE OPTIC TRACT BY FOCUSED ULTRASOUND

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The use of focused ultrasound (FUS) to obtain reversible changes in the CNS is useful both in experimental physiology and in medical practice. For example, during ultrasonic neurosurgical operations reversible (temporary) blocking of an area of brain tissue could serve as a function test for settling the issue of whether final (irreversible) destruction of a given structure is acceptable depending on the result obtained.

It has been shown that reversible blocking of some brain formations by FUS is possible in principle [4, 5]. The doses required for functional blocking of nerve structures were found to be so close to destructive doses that the use of this procedure in practice seemed to be contraindicated.

The writers have studied the possibility of using irradiation with FUS under conditions known to be nondestructive in order to produce changes leading to the temporary, reversible, blocking of conduction of information along the optic tract. Evoked potentials (EPs) of the brain centers of the visual system were used as the indicator of functional changes.

To verify the functional character of the action of the chosen doses of FUS, an electron-microscopic investigation was made of endings of the optic tract (OT) fibers in the superior colliculus (SC).

EXPERIMENTAL METHOD

The experimental apparatus enabled FUS to be applied to the optic tract and, at the same time, electrical activity from different parts of the visual system to be recorded [1–3].

Experiments were carried out on 40 adult cats anesthetized with pentobarbital. Ultrasound was applied through a burr-hole above the part of the brain to be irradiated, and the uninjured dura mater. A cannula, consisting of a hollow cylinder made of transparent plastic (Fig. 1), filled with physiological saline 2 heated to 37°C, was fixed hermetically to the animal's head above the burr-hole. The center of the focal region of the generator 3 was made to coincide with the test structure 4 by means of a coordinate system. The coordinates

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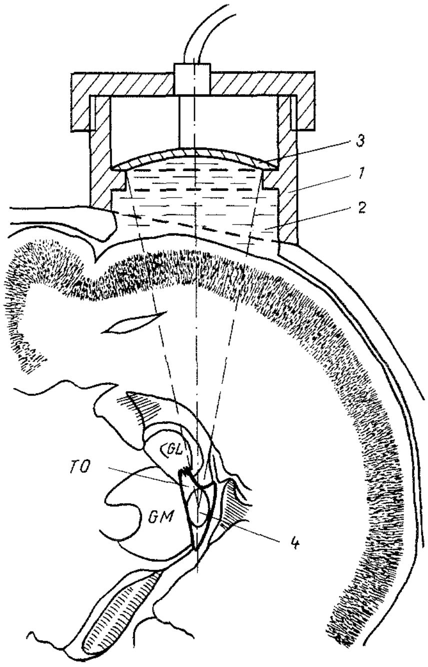

Fig. 1. Scheme of apparatus for local application of focused ultrasound to cat's optic tract: 1) cannula, 2) physiological saline, 3) focusing ultrasonic generator, 4) center of focal region of generator.

of the center of the part of the optic tract to be irradiated were determined by means of Jasper and Ajmone-Marsan's atlas [5]. To monitor the blocking effect, electrodes were implanted in the optic tract and occipital cortex. A focusing generator with resonance frequency of 0.975 MHz, focal distance of 2.5 cm, and plate 1.8 cm in diameter were used. The apparatus included an electrophysiological system designed to record EPs and photic stimulation of the experimental animal's retina.

The experimental procedure consisted of applying ultrasound in the form of successive pulses of assigned duration and frequency to the structure chosen for irradiation. The retina was illuminated with flashes of light at the same frequency, but with a time lag relative to the ultrasonic pulse. By this method the beginning of the change in bioelectrical activity of the structure tested could be identified precisely.

Mainly a pulsed ultrasonic current was used. The intensity, averaged over the area of the focal region, ranged from 7 to 63 W/cm^2, the pulse duration from 5 to 50 msec, the pulse repetition frequency from 0.5 to 50 Hz, and the duration of the irradiation procedure from 10 sec to 1 min. For the electron-microscopic study the animals were perfused with a mixture of 1.25% glutaraldehyde solution and 1% paraformaldehyde solution in phosphate buffer (pH 7.2–7.4). Pieces of tissue from the superficial layers of SC were cut in the frontal plane, fixed with 1% OsO_4 solution, dehydrated, and embedded in a mixture of Epon and Araldite. Semithin and ultrathin sections were cut on an LKM ultramicrotome. The sections were stained on grids with uranyl acetate and lead citrate and examined in the NI–11E electron microscope.

EXPERIMENTAL RESULTS

Under the influence of FUS on the region of the optic tract, changes in amplitude and temporal parameters of EP were observed in the latter. The character and duration of the changes were determined by parameters of the dose of irradiation and varied widely when these were altered. Inhibition of EPs could be complete, partial, or variable. The term variable implies a change in EP when the mean values of the amplitude and the other parameters were preserved but the dispersion of these values was much wider than normally. According to the criterion of recovery, the effects were completely or partially reversible or irreversible. In certain cases, instead of inhibition of EP, its amplitude was temporarily increased. Different combinations of the effects could be observed.

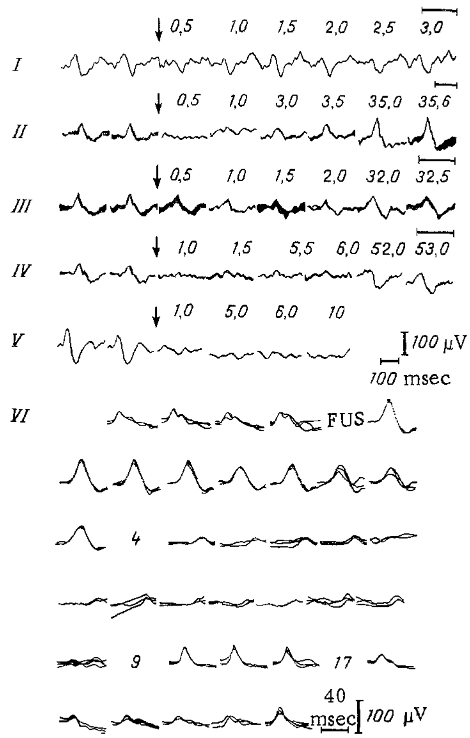

Fig. 2. Response of optic tract to FUS. On left of arrows – EPs of optic tract in response to flashes before application of ultrasound; after arrows – at end of application of FUS. Numbers denote time (in min) after end of application of FUS. I) Variable changes; II, IV) completely reversible inhibition; III) partial inhibition, V) irreversible inhibition, VI) changes in EP in visual cortex during complete reversible blocking of electrical activity of optic tract by FUS.

Some examples of responses of the optic tract to FUS (pulsed) are given in Fig. 2: I) variable change in EP observed under the following conditions of application of ultrasound (US): intensity of US averaged over area of focal region I = 7 W/cm^2, pulse duration t= 30 msec, pulse repetition frequency f = 10 Hz, total duration of irradiation T = 20 sec; II) complete inhibition of EP followed by complete recovery of all phases 5 min after end of irradiation (I = 25 W/cm^2, t = 50 msec, f = 10 Hz, T = 40 sec); III) partial inhibition of electrical activity of optic tract, expressed as a temporary decrease in amplitude of phases of the EP (I = 7 W/cm^2, t = 30 msec, f = 20 Hz, T = 1 min); IV) complete inhibition of EP followed by recovery (I = 7 W/cm^2, t = 30 msec, f = 10 Hz, T = 1 min); V) complete irreversible inhibition of EP (I = 63 W/cm^2, t = 10 msec, f = 50 Hz, T = 1 min).

In the cases examined above the effect of inhibition of EP in the optic tract thus appeared 10–60 sec after the time of application of FUS. Complete blocking of conduction in the optic tract lasted from a few seconds to a few minutes, but sometimes final recovery of EP was not observed until after 1 h. Data (Fig. 2, II, IV) showing that temporary inhibition of EP can be obtained by means of FUS, followed by complete recovery of its shape and amplitude, are of the greatest practical interest. When the dynamics of changes in EP in the visual cortex was studied during complete reversible inhibition of conduction in the optic tract under the influence of FUS, in some cases effects similar to those described above were recorded. Sometimes, however, the effect did not appear until after a delay of 4–5 min compared with this parameter in the optic tract, although the time course of the process was the same. In some cases inhibition of EP was preceded by a transient increase in its amplitude. An example of this effect is given in Fig. 2, VI. After application of FUS a considerable increase in amplitude of EP was observed in the visual cortex, and after 4 min this was replaced by total inhibition of bioelectrical activity. EP recovered after 9 min, when its amplitude was a little higher than that observed before irradiation. Evoked activity did not recover until 17 min after the end of application of ultrasound.

Temporary inhibition of EP followed by recovery of its shape and amplitude could thus be obtained by means of FUS.

To study the character of the ultrastructural changes produced by the different conditions of ultrasonic irradiation, endings of optic tract fibers (optic terminals, OT) were investigated in SC 20 min–1 h after the end of application of ultrasound, i.e., in the period of recovery of electrical activity in the optic tract.

Terminals containing round synaptic vesicles and mitochondria with a pale matrix and a few cristae were identified previously as optic. From 20 min to 1 h after ultrasonic irradiation a reduction in the number of synaptic vesicles was observed in a small proporation of OT compared with normal, together with the appearance of vacuole-like inclusions. In some large OT there was an appreciable increase in the number of synaptic vesicles; as well as round vesicles, flattened vesicles also appeared, and osmiophilia of the matrix of OT increased. In some endings, with a diameter of 2 μ, hyperproduction of mitochondria was observed, whereas in other terminals, with an increased number of synaptic vesicles, destruction of individual mitochondria could be observed. In postsynaptic components of synapses formed by these terminals, no visible ultrastructural changes were present. In the opposite hemisphere no marked structural changes could be detected in OT. The ultrastructure of synapses formed by terminals of nonoptic origin in SC on the ipsilateral and contralateral sides likewise remained unaffected.

The possibility of using FUS to obtain reversible changes in brain structures was thus investigated. In particular, the effect of reversible functional blocking of the optic tract and blocking of conduction of visual information through the action of FUS on the optic tract was reliably recorded. The study of the ultrastructure of endings of optic tract fibers in SC showed that in the period of recovery of electrical activity after complete reversible blockade of the optic tract, the changes observed were reversible in character, and expressed mainly as changes in the number of synaptic vesicles and in their shape, and also changes in the state of the mitochondria.

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