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Early ultrasonic effects upon mammalian CNS structures (chemical synapses)

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(Received 11 November 1980; accepted for publication 10 February 1981)

It has been found that ultrasonic effects upon mammalian CNS chemical synapses are among the earliest morphologic changes that occur. This provides a possible explanation for the earlier findings which showed that a functional effect can be recorded almost immediately following certain ultrasonic exposures while no morphological abnormalities were observed with the light microscope.

PACS numbers: 43.80.Gx, 43.35.Wa

INTRODUCTION

It has been known for some time that under certain conditions ultrasound produces nearly instantaneous functional changes to the mammalian central nervous system (CNS), including reversible suppression of nerve potentials[1] and irreversible limb paralysis.[2] However, it has also been found from light microscopy studies that the earliest morphological changes do not become observable until approximately 10 min. after exposure.[3] For these reasons it had been postulated that ultrasound affects biological structures too small to be seen in the light microscope and that approximately 10 min. of physiological functioning is required for the morphologic changes to involve dimensions sufficient to allow observation at the light microscope level.[4] These findings provoked ultrastructural studies with the electron microscope (EM) and reports of effects upon mitochondria,[5,6] nuclei,[6] endoplasmic reticulm,[5,6] attached ribosomes,[5] lysosomes,[6] and other features of the fine structure in biological tissues. These EM studies employed lesser intensities and much longer exposure times than those used in the present study where the tissue is irradiated with a focused sound beam of SPTA intensity of 300 W/cm^2 for exposure times of 0.5 to 3 s. Post-exposure sacrifice time is varied from immediately following and up to 15 min. after the exposure in an attempt to record the evolution of observed altered morphology.

Although many structures within the exposed tissue volume exhibited abnormal morphology, e.g., mitochondria, myelin, endoplasmic reticulum, nuclei, etc., it is the purpose of this note to report the observation of direct and almost immediate effects upon the chemical synapse. This synaptic damage may result in a loss of function for this structure, and probably explains the immediate irreversible response of mammalian CNS function to ultrasound mentioned above.

I. METHODS

The procedure for preparing the specimens (adult female cat) for the ultrasonic exposure has been described in detail previously.[3] Briefly, the animal is anesthetized with Diabutol and placed in a Horsley–Clark stereotaxic apparatus. A bone flap is removed from the area over the cerebrum, but the *dura mater* is left intact. A headpan is attached to the animal, using the skin around the cranial incision to form a watertight seal, and is filled with degassed physiologic saline. The saline, brain, and body temperatures are regulated to 37°C. Three regions of each brain are selected for exposure with each successive exposure being made in opposite hemispheres. The corresponding sites in the brain hemisphere not receiving the ultrasonic exposure are sham irradiated. The exposure conditions involved delivering a temporally rectangular envelope of 1 MHz ultrasound at 300 W/cm^2 to the selected sites for exposure times ranging from 0.5 to 3 s.

A procedure was developed to provide for a quick perfusion of the brain following the last ultrasonic exposure of each animal. The right common carotid artery was cannulated in each specimen during preparation prior to the first exposure. Following the last exposure, the left carotid artery was occluded, both common jugular veins were severed, and perfusion with physiologic saline was initiated within 20 s, via the cannulated artery. Saline perfusion was continued for 2–3 min, followed by perfusion for 1.5 to 2 h by 800–1000 ml of a 2.5% gluteraldehyde—2.0% paraformaldehyde solution in a 0.1 M cacodylate buffer (pH 7.2 at 37°C). The perfusion was carried out using a syringe to drive the solutions through the cannula. Measurements made with a Statham pressure transducer connected to a cannula inserted in the right caro-

tid artery to record the mean atrial pressure prior to and during the perfusion procedure yielded, respectively, 81 and 65 mm Hg (peak pressure not exceeding 70 mm Hg), confirming that the syringe-driven perfusion did not produce abnormally high pressures in the animal that could have resulted in ultrastructural artifacts.

A hollow tube attached to the stereotaxic device was used to obtain cylindrical cores of tissue containing the exposed and control tissue volumes. Disks containing these tissue volumes were cut from the cores using a tissue slicer.[7] The tissue was routinely dehydrated and embedded for transmission electron microscopy (TEM) and examined with a JEOL JEM-100C TEM with accelerating voltages of 80 and 100 kV.

II. RESULTS

A tissue section of subcortical gray matter from a sham irradiated site is shown in Fig. 1 exhibiting chemical synapses, mitochondria, and other structures. Several distinct types of chemical synapses are seen[8–10] which have several basic features in common. These synapses have pre- and post-synaptic elements separated by a cleft 200–400 Å wide. An electron-dense material, termed the junctional density, is associated with the synaptic region. There is evidence to suggest that this junctional density has a high tubulin content.[10] The presynaptic element, or bouton, contains small membranous vesicles which have variable size, shape, and electron density, depending upon the type of synapse they occupy. Some of the synaptic vesicles are intimately associated with the junctional density, and this relationship is referred to as the synaptic complex.[8] The remaining vesicles within the bouton are apparently distributed randomly. The bound regions seen in the figure containing vesicles but without junctional density are boutons for which the section plane does not contain the synaptic complex.

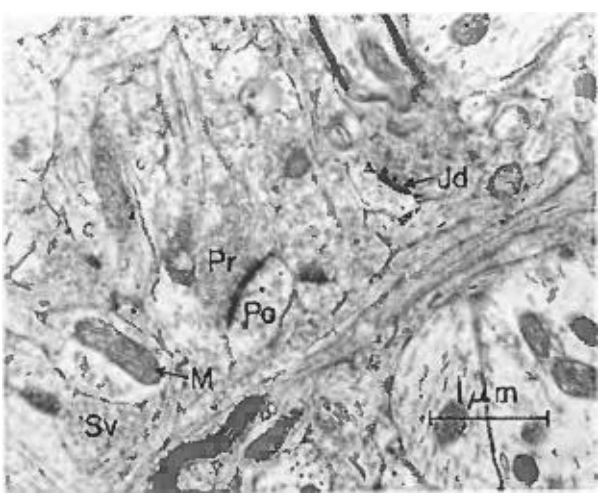

FIG. 1. Gray matter from control site. Synaptic complexes (><) are distinguished by the junctional density (Jd) which is denser on the postsynaptic (Po) than the presynaptic (Pr) side of the cleft. The bouton contains the synaptic vesicles (Sv) and occasionally mitochondria (M).

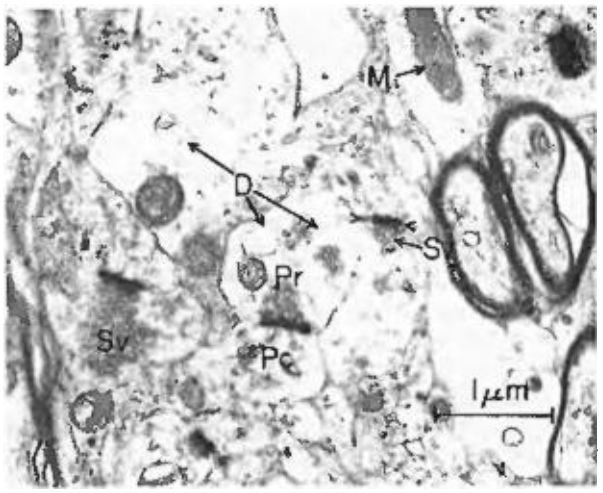

FIG. 2. Gray matter from exposed site, 300 W/cm² for 1.2 s and aldehyde perfusion initiated 2 min. after the exposure. Notice debris (D) in the presynaptic element (Pr) and the decrease in the number of synaptic vesicles (Sv). In several presynaptic elements the vesicles appear clumped, especially in the vicinity of the synaptic complex (><). Only subtle effects are seen in the mitochondria (M).

Figure 2 shows a section of tissue having experienced an ultrasonic exposure of 300 W/cm² for 1.2 s. The saline flushing was initiated 30 s after the exposure and the first introduction of the aldehyde solution occurred within 2 min. It is clear that synapses have been altered morphologically and that the degree of damage varies among these chemical synapses present in the micrograph, some appearing almost normal. The number of synaptic vesicles is decreased in the affected structures and the remaining vesicles tend to clump together. The electron density of the cytoplasm within the bouton is less than that of the sham irradiated sections, and the pre- and post-synaptic elements appear to be swollen. A decrease in the electron density of the junctional density is also evident. Amorphous debris is observed both within the bouton and associated with the clumped synaptic vesicles. The appearance of some of this debris suggests that it may be deformed synaptic vesicles. Some debris is also visible within the postsynaptic element. The more damaged structures have very few synaptic vesicles, and the junctional density is sparse and irregularly shaped, as opposed to the smooth distribution seen in the control tissue. More membranous debris is also observed, and the width of the synaptic cleft has increased 10%–25%. Similar synaptic damage has been observed in tissue receiving exposures as short as 0.5 s, but a smaller number of the synapses are affected.

In tissue receiving an exposure longer than 1.2 s, a greater proportion of the synapses were affected and a greater number of these exhibited more extensive damage, than specimens irradiate for shorter periods. With a given exposure and increasing post-exposure sacrifice time, the electron density of the axoplasm and junctional density are observed to decrease, and the average size of the pre- and post-synaptic elements

increases. The distribution of the junctional density becomes more irregular and the synaptic cleft is, on the average, noticeably wider. All synaptic types appear to respond in a similar manner to the ultrasound exposures.

III. DISCUSSION

The observed morphologic changes in the synapses are considered to explain the irreversible interruption in CNS functioning following ultrasonic exposure of appropriate conditions. It should be noted that the mitochondria in the micrograph seen in Fig. 2 exhibit little, if any, sign of altered morphology, while the synapses are distinctly altered. This observation suggests that the synapses are more sensitive to ultrasonic exposure than the mitochondria, which have been regarded as the most sensitive structures to ultrasound.[5] Details of these findings and those of other microstructures will be the subject of a more complete reporting.

ACKNOWLEDGMENTS

The authors acknowledge gratefully the partial support of this research by a grant from the National Science Foundation (ENG 77-20934). M. J. B. has been partially supported by a National Cancer Institute Research Service Award (CA 9067). The authors acknowledge, with thanks, discussions with Dr. B. Jakstys and Dr. A. Kokko-Cunningham regarding interpretation of micrographs.

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Comments on impedance tube measurements

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(Received 5 December 1980; accepted for publication 2 January 1981)

The application of a new impedance measuring method to water-filled tubes is discussed. It is shown that source reflections do not invalidate the method, and that valid results can be obtained at frequencies several times higher than that of the first cross mode of the tube.

PACS numbers: 43.85.Bh

Recent papers[1,2] have described a new method of measuring the impedance $Z(\omega)$ of a sample in an impedance tube over a wide frequency (ω) range.

In the new method, the source radiates a wideband of random noise, and the signals from two fixed pressure pick ups are processed to yield $Z(\omega)$; the results are available in a few seconds. This contrasts with the traditional methods: In air, the maxima and minima of a standing wave pattern are measured by a movable pick up; in water, the amplitude and phase of a reflected pulse of sine waves are measured. In both cases the measurements must be made separately at each frequency required, which takes much longer than the new method.

The papers[1,2] give experimental results obtained using the new method in impedance tubes containing air, and the question arises as to how well the new method will work for tubes containing water. In this connection the following two points may be made.

(1) Will repeated reflections of the signal from the sound source upset the results? In air, the source is generally a paper-cone loud speaker, which may give little reflection of the signal. In water, however, the source will typically be a good reflector.

The following argument shows that such reflections will not invalidate the results of the new method.

The signal radiated by the source is a broadband of