ABSTRACT

Effect of low-level microwave irradiation on the uptake of horseradish peroxidase by synaptosomes.

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Synaptosomes isolated from whole rat brain were incubated at 30°C in physiological saline medium which contained horseradish peroxidase (HRP), an extracellular tracer. Synaptosome preparations were either incubated for 70 minutes in 5 mM K^+ medium (control), deploarized by incubation in 50 mM K^+ medium for 30 minutes, or irradiated at 960 MHz (absorbed power ~ 1.5 mW/g) for 30 minutes in 5 mM K^+ medium. K^+ stimulation and irradiation were both followed by a 40 minute "rest" in 5 mM K^+ medium. The synaptosomes were then fixed, incubated with H_2O_2 and diaminobenzidine, and prepared for electron microscopy. Thin sections were examined and photographed at random.

There is a significantly higher frequency of synaptosome profiles with 2 or more HRP-labeled vesicles and vacuoles in the K^+ depolarized preparations. There is a small nonsignificant difference between the irradiated and control preparations, the irradiated synaptosomes showing a slightly higher level of uptake than the controls.

Synaptosomes, the pinched-off presynaptic terminals of neurons, have many of the characteristics of intact nerve terminals, such as the release of transmitters upon depolarization in the presence of Ca^{2+}. Like other nerve terminals, they also have been demonstrated to exhibit "membrane recycling,"[1] a process whereby synaptic vesicles lost during transmitter release are replaced by vesicles pinched off the terminal membrane; when a tracer such as horseradish peroxidase (HRP) is present in the external medium, it will be taken up into these newly-formed vesicles.

Synaptosomes were prepared by the method of Hajos[2] from one whole rat brain. After isolation the synaptosome suspension was slowly diluted with about 2 volumes of Ca-free Na^+5K[3] at 4°C over about 15 minutes, then centrifuged at 7000 g for 5 minutes. The synaptosome fraction was resuspended in 0.5 ml Na^+5K at a concentration of about 2-4 mg protein/ml; the Na^+5K solution also contained HRP (Sigma type VI previously dialized against Na^+5K) at a concentration of 10 mg/ml. The synaptosome solution was then incubated at 30°C for 10 minutes to allow the synaptosomes to develop a membrane potential, after which they were either depolarized using high K^+ concentrations, or irradiated: 1) K^+ depolarized synaptosomes: after the initial incubation period, 0.5 ml of Na^+95K was added to the incubation medium (to give a final K^+ concentration of 50 mM). After 30 minutes incubation, the tissue was allowed to settle and most of this medium drawn off and replaced with Na^+5K, also containing HRP. After 40 minutes further incubation, fixative was added. 2) Microwave-irradiated synaptosomes: 0.5 ml Na^+5K was added and the synaptosome suspension was irradiated at 960 MHz for 30 minutes, then allowed to incubate an additional 40 minutes before fixative was added. A circulating bath kept the temperature at 30°C. Absorbed power was about 1.5 mW/g synaptosome suspension. 3) Controls were run for both the K^+ stimulated and irradiated preparations. 0.5 ml Na^+5K was added and the synaptosomes incubated at 30°C for 70 minutes.

The synaptosomes were fixed overnight at 4°C in Karnovsky's fixative[4]. They were then pelleted, washed in 0.1 M cacodylate buffer, (pH 7.2) and incubated in a solution of 3% diaminobenzidine in 0.02 M Tris buffer (pH 7.6) and 0.01% H_2O_2 for 1 hour, after which the pellets were stained for 2 hours with ferrocyanide-reduced osmium tetroxide[5], dehydrated in alcohols and propylene oxide, and embedded in Epon.

Thin sections of fairly uniform thickness (silver-gray) were examined under an electron microscope and photographed at random. The identity of the pellets (whether experimental or control) was unknown at the time the micrographs were taken as well as when counts of labeled vesicles were made.

The micrographs were examined and the number of synaptosome profiles containing a given number of HRP-labeled inclusions was tallied. Profiles of an average diameter of less than 0.4 μm or containing less than 5 vesicles total (labeled or unlabeled) were not included in the counts; neither were profiles in which HRP had entered the cytoplasm, indicating damage to the synaptosomal membrane. Labeled inclusion of any size (whether the smaller "vesicles" of 50-90 nm diameter or the larger, sometimes irregularly shaped "vacuoles" or "cisternae") were counted.

K^+ depolarized synaptosomes showed a significantly higher frequency of synaptosomes with 2 or more labeled vesicles and vacuoles (see table.) Profiles with very large numbers of labeled inclusions (15 to 18 in one profile) are seen infrequently in depolarized preparations but to date have not been seen in control preparations; only one profile with more than 8 labeled vesicles or vacuoles has been found in a control preparation, out of 231 profiles examined, compared to 17 profiles out of 329 profiles examined in K^+ depolarized preparations.

Microwave-irradiated preparations show no significant difference from control preparations. However, profiles with very large numbers of labeled vesicles and vacuoles (up to 18) are occasionally seen. In the one experiment done to date, 8 out of 234 profiles had more than 8 labeled inclusions. Whether this trend will be consistent remains to be seen.

Treatment	Number of synaptosome profiles examined	Percentage of profiles with ≥ : HRP-labeled vesicles and vacuoles					
		1	2	4	6	8	10
30 min. 50 mM K5 stimulation plus 40 min. "rest"	110	65	47**	19*	9	4	3
K^+ stimulation control	71	49	24	4	0	0	0
30 min. 960 MHz irradiation plus 40 min. "rest"	234	64	42	15	6	3	2
irradiation control	325	66	37	13	4	0	0

All four treatments were done at the same time, using synaptosomes from the same preparation. Controls were run for both K^+ stimulated and irradiated treatments because these used different temperature baths which may have been a few fractions of a degree different from each other.

*Significantly different from control values at $P < 0.01$ by chi square analysis.

**Significantly different at $P < 0.005$.

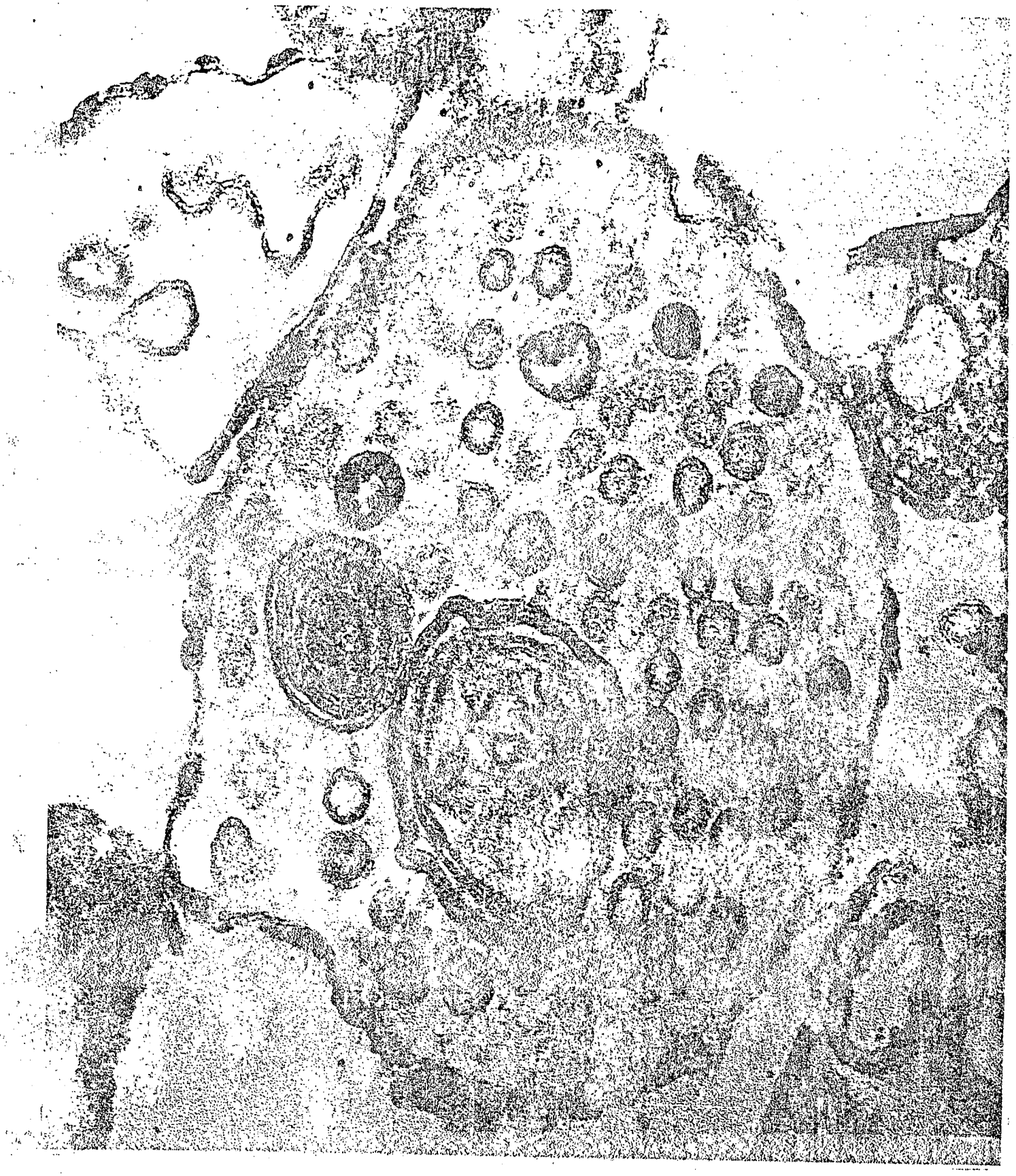
[1]Fried, R. C. and Blaustein, M. P. (1978). J. Cell Biol. 78:685-700.

[2]Hajos, F. and Csillag, A. (1976). Brain Res. 112:207-213.

[3]The composition of the physiological salines used was as follows: Na^+5K contained 132 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 1.3 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 10 mM glucose and 20 mM Tris base, titrated to pH 7.6 with maleic acid. Ca-free Na^+5K was the same except that $CaCl_2$ was omitted; Na^+95K contained 95 mM KCl and 42 mM NaCl.

[4]Karnovsky, M. J. (1965). J. Cell Biol. 272:137a.

[5]Karnovsky, M. J. (1971). Proceedings of the 11th Annual Meeting of the American Society for Cell Biology 27:146a.



K^+ depolarized synaptosome with HRP-labeled vesicles ~ 190,000X