# IN VITRO ACETYLCHOLINE SYNTHESIS AND RELEASE IN RAJA ERINACEA ELECTRIC ORGAN

Oliver M. Brown
Pharmacology Department, State University of New York,
Health Science Center, Syracuse, New York 13210

The skate, Raja erinacea, has weak electric organs in the tail, one on each side of the spinal cord. These organs, with the ampullae of Lorenzini as detectors, may serve the fish as a navagation mechanism (Lissmann, J. Exptl. Biol. 35: 156-191, 1958). The electrical activity of this organ has received some attention (Bennett, Ann. N.Y. Acad. Sci. 94:458-509, 1961), and although it is thought to be a purely cholinergic organ, the neurotransmitter biochemistry of skate electric organ has not been described. This is a preliminary report of efforts to characterize the electrical properties and the dynamics of the neurotransmitter, acetylcholine (ACh), in this tissue. It is further hoped that this organ will serve as a valuable model system to study the mechanisms of ACh synthesis and release. A vigorous and growing controversy concerning the synaptic vesicle hypothesis of neurotransmitter release (Dunant and Israel, Sci. Amer. 252:58-66, 1985) indicates the need to re-examine these mechanisms in such a model tissue.

METHOD Skates of both sexes (appx. 1 kg) were sacrificed by a blow to the head, and the electric tissue was dissected from both lateral aspects of the tail and placed in elasmobranch buffer (Dunant, et al., J. Neurochem. 27:975-977, 1976). For electrical stimulation, sections of usually 2 cm in length (typical diameter was 3 mm) were placed over stainless steel electrodes spaced every 0.5 cm in either of two chambers: 1) a horizontal nerve conduction chamber, tissue was not in contact with buffer, chamber rested on a bed of ice; or 2) an inclined drip-flow superfusion chamber which was cast from molten wax (poured over PE tubing which served as a cooling line for gravity-flow ice water), and carved to create an electrode/tissue chamber, cooled buffer was constantly dripped onto and flowed over the length of the tissue, and was collected for analysis. Tissue sections were kept at 15-19° C and were stimulated from end to end with a Grass stimulator; electrical events over the central 1 cm were monitored on a Tectronix oscilloscope. Various stimulation parameters were used: voltage from 8 to 30 v, frequency from 2 to 20 Hz, and all stimulus pulses were of 0.1 msec duration. Samples were stimulated to various degrees of electrical fatigue and fixed and stored for later ACh analysis by plunging into acetonitrile and freezing; some samples were also fixed for examination by electron microscopy. For ACh release experiments, tissue sections were pre-stimulated and incubated in elasmobranch buffer containing labeled choline (Ch) precursor: either 0.38 $\mu$M $^{3}$H-Ch (30 $\mu$Ci) or, 50 $\mu$M $D_4$-Ch. Tritiated samples were counted by liquid scintillation. Deuterated and non-deuterated Ch and ACh from tissue and from superfusate were analyzed by pyrolysis-gas chromatography-mass spectrometry (Brown and Salata, Life Sci. 33:213-224, 1983).

RESULTS Electrical stimulation of Raja electric organ sections resulted in an electrical discharge of approximately 150-250 mv per cm. Fig. 1 illustrates an example of such a discharge, resulting from a 20 v, 0.1 msec electrical stimulus; the early down-stroke in the figure is the stimulation artifact, and the up-stroke is the electrical discharge from the tissue. Using supramaximal stimulation voltages, the electric organ demonstrated fatigue of electrical discharge amplitude with time. This fatigue is demonstrated by the oscilloscope tracings shown in Fig. 2. The curves represent tissue discharge at 1, 2, 3, 4, and 6 min from a sample stimulated with 20 v, 0.1 msec pulses at a frequency of 20 Hz.


FIG. 1

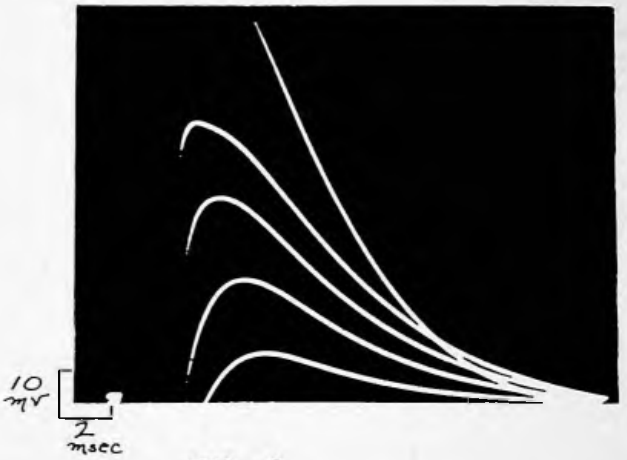


FIG. 2

The rate of electrical discharge fatigue was found to be exponential, and dependant on stimulation frequency. Figure 3 shows the typical fade in amplitude with time, in this case for three tissue sections (from the same electric organ) that were stimulated with 25 v at 20, 10 or 2 Hz (mv normalized for comparison). The half-times for fatigue of electrical discharge in this experiment were 2 min at 20 Hz, 11 min at 10 Hz, and 19 min at 2 Hz. Recovery of the tissue was also examined, with most samples recovering some discharge activity within seconds of cessation of stimulation, and recovery to approximately 80% of original amplitude within 15-30 min.

Some experiments were performed to measure the release of ACh with stimulation. Tissue sections were electrically stimulated to near fatigue and allowed to recover in elasmobranch buffer containing Ch labeled with either deuterium or tritium. Labeled tissue sections were placed in the drip-flow chamber and superfused with buffer. Superfusate was collected for 2 min periods during several cycles of electrical stimulation and rest. The results of a typical experiment are shown in Fig. 4. This tissue sample was pre-stimulated and incubated for 2 hr in buffer containing 0.38 uM tritiated Ch, then washed in unlabeled buffer for 1 hr. Plotted are the cpm for each 2 min superfusate collection against time. Electrical stimuli of 20 v at 20 Hz were applied for 2 min at the times indicated by the bars on the graph.

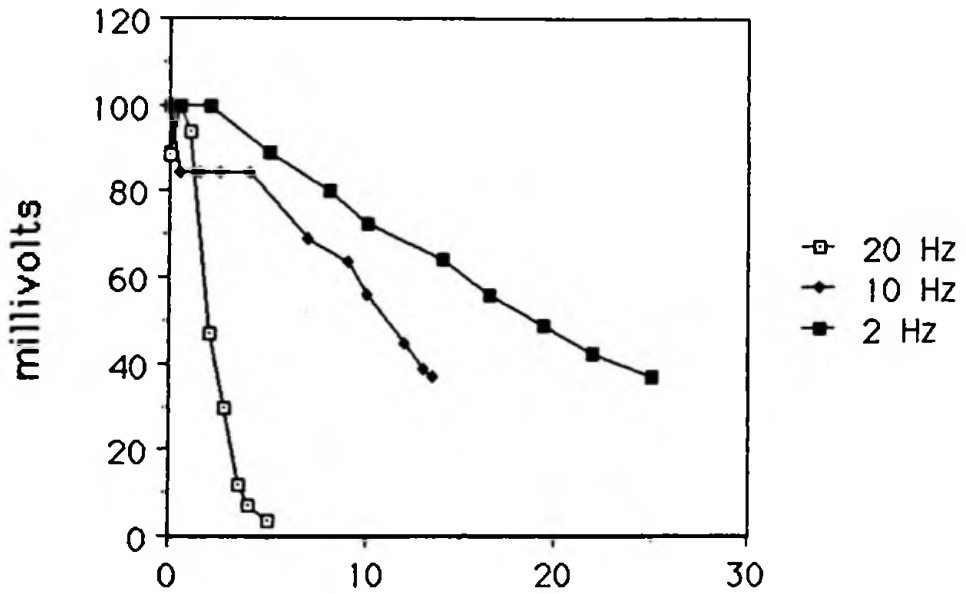
120
100
80
60
40
20
0
millivolts
0
10
20
30
20 Hz
10 Hz
2 Hz

Fig. 3

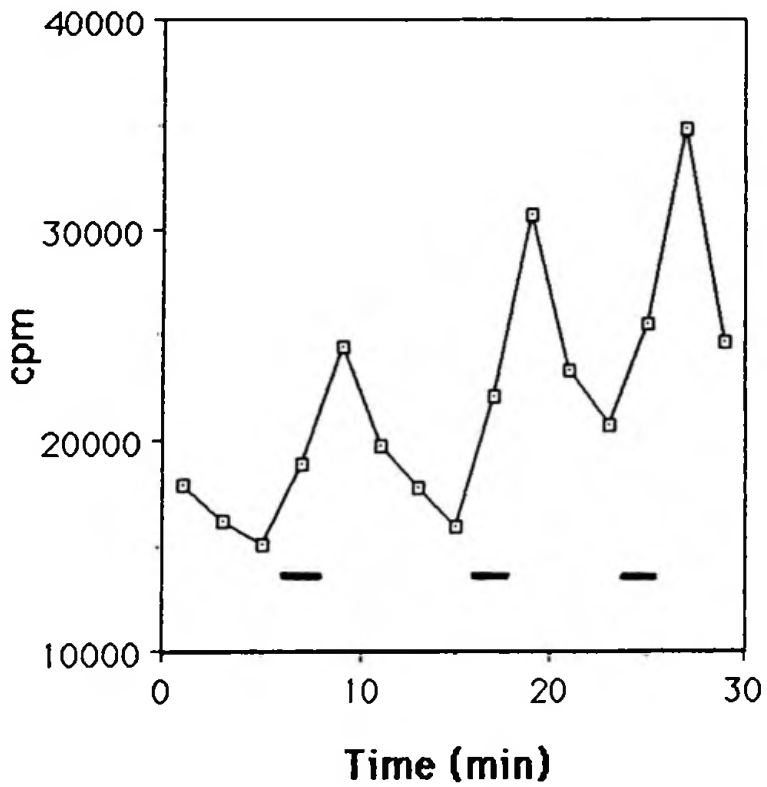
40000
30000
20000
10000
cpm
0
10
20
30
Time (min)

Fig. 4

Stimulation resulted in the release of tritium, presumably as ACh. This experiment demonstrates that the tissue and the method are useful to study the uptake of Ch as an ACh precursor, the synthesis of ACh, and the release of ACh with electrical stimulation.

CONCLUSION The electric organ of the skate, Raja erinacea is a very promising model system for the study of neurotransmitter dynamics and release mechanisms. In this preliminary study many technical aspects of working with this unique tissue were developed: dissection and handling, temperature requirements, incubation conditions, stimulation parameters, recovery conditions, and a custom tissue chamber was constructed (which allows drip-flow superfusion, temperature control and electrical stimulation). The electrical properties of the tissue were characterized. The uptake of labeled Ch, and synthesis and release of ACh were demonstrated. Characterization of ACh dynamics in this tissue by mass spectrometric analysis is in progress.

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