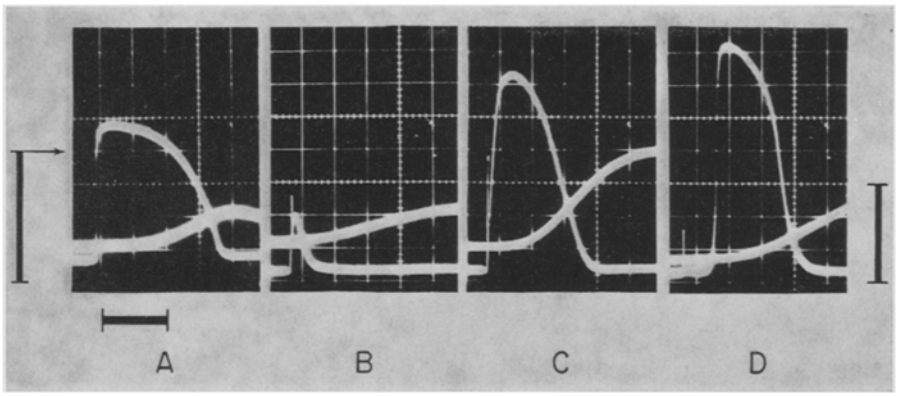

Electrical and mechanical responses of frog atrium in Na-free high Ca (83 mM) solution in presence of epinephrine ($5\times10^{-6}M$) and TTX (10^{-7} g/ml). A) Control in Ringer *tris* solution; B) and C) local responses; D) conducted response. O· reference is indicated by arrow; left vertical bar: 100 mV; right vertical bar 0.25 g; horizontal bar: 200 msec.

duction to take place. Furthermore, the fact that epinephrine increases the magnitude and duration of the potentials strongly supports the assumption that the drug increases P_{Ca} as concluded from voltage clamp experiments (REUTER[3], VASSORT et al.[6]).

The change in membrane potential during conduction permits conclusions to be drawn regarding the internal Ca^{++} concentration. If a maximal value of 90 mV for the overshoot is taken as the equilibrium potential of Ca, the internal concentration at the beginning of contraction, calculated from the Nernst equation, is $6.5\times10^{-5}M$. However, as it is unlikely that an equilibrium for Ca is reached, this value must be considered merely as a maximal value. Thus, the results suggests that intracellular Ca^{++} concentration remains low inspite of the high concentration in the medium.

Résumé. L'activité électrique de préparations atriales de grenouille soumises à un milieu de Ringer dont tout le Na^{+} est remplacé par du Ca^{++} a été enregistrée à l'aide de microélectrodes. Dans ces conditions, il a été possible d'obtenir des potentiels d'action propagés. Ces réponses sont caractérisées par une très grande inversion de potentiel dont l'amplitude, augmentée par la présence d'adrénaline dans le milieu, peut atteindre 90 mV pour une dépolarisation totale de 185 mV, du fait d'une hyperpolarisation de la membrane. Ces résultats démontrent clairement le rôle du Ca^{++} dans l'activité électrique cardiaque.

J. F. DELAHAYES[7]

Department of Physiology, Ohio State University, 333 West 10th Avenue, Columbus (Ohio 43210, USA),
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Vesicle Hypothesis: Effect of Nerve Stimulation on the Synaptic Vesicles of Motor Endplates

Ever since they were first described, the synaptic vesicles of axon terminals have been regarded as the morphological correlate of quantal transmitter release. This so-called 'vesicle hypothesis' has become almost axiomatic in the neuro-sciences.

To test the hypothesis, various attempts have been made to find whether the concentration of synaptic vesicles is altered by experimental conditions which influence either transmitter release or synthesis. The results seem somewhat confusing. Thus, some authors have reported decreased number of vesicles following stimulation[1–3], whereas others have found an increase[1,4–5]. Exposure to hemicholinium alone[6] has resulted in decreased number of vesicles, and hemicholinium combined with stimulation has caused a decrease in vesicles[4–5] or no change[7].

The present experiments were performed with a similar purpose to those just referred to. Distinctive features of our experiments are firstly that the stimulation of the nerve outlasted fixation of the muscle and the nerve terminals, and further, that the stimulation frequencies were so high as to put an exhaustive load on the presynaptic structures.

Methods. Phrenic nerve-diaphragm preparations from male 200 g albino rats were removed under ether anesthesia, fixed by threads to a glass fork, and placed horizontally in Tyrode solution[8] at 37 °C, carbogen bubbling through the solution. The phrenic nerve was stimulated by supramaximal square wave pulses of 0.1 ms width, contraction observed visually. Towards the end of the stimulation period the fork was carefully transferred to the surface of the fixing solution (2% paraformaldehyde + 2% glutaraldehyde in isotonic phosphate buffer[9], pH 7.3), the muscle being fixed from the abdominal side. In the course of 2–3 min, the diaphragm was lowered gradually into the fixation medium until it was completely

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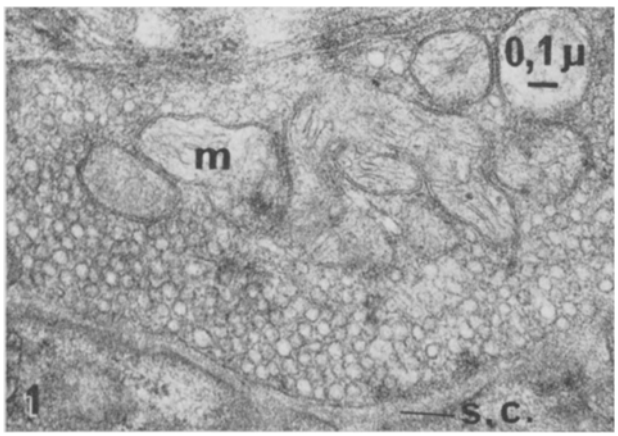

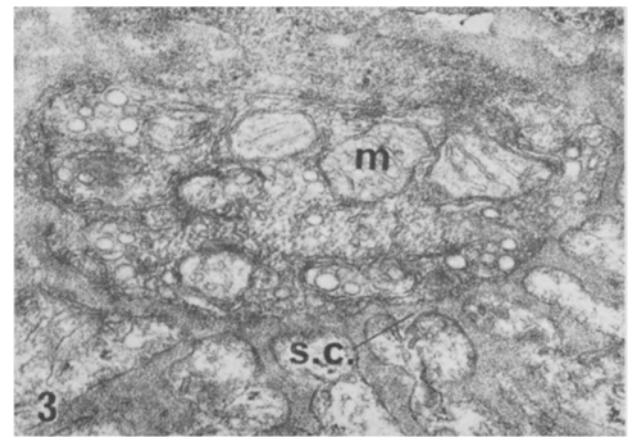

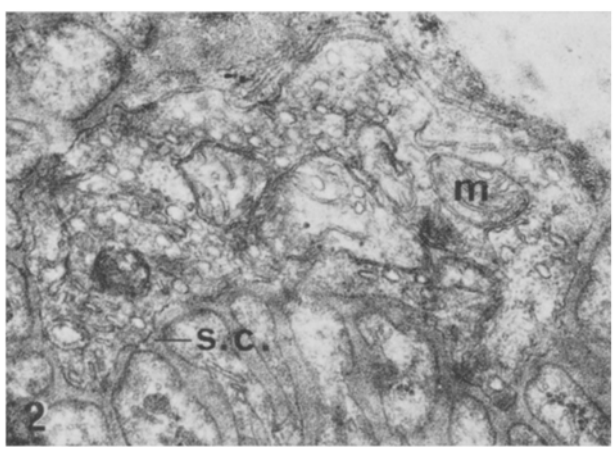

Fig. 1. Shows a normal control endplate, whereas Figures 2 and 3 are endplates from the same hemidiaphragm, submerged into the fixative after 20 sec stimulation at 100 Hz, stimulation being continued for another 3 min. A marked reduction in the number of synaptic vesicles from Figure 1 through Figures 2 and 3 is evident. Note also the increased number of elongated vesicle profiles in Figure 2. (m, mitochondrion; s.c., synaptic cleft.)

submerged, the stimulation being maintained for another 3 min. 5 experiments were carried out, the preparations being stimulated at 100 Hz for 20 sec, and 50 Hz for 2, 5, 5, and 10 min before onset of fixation. Normal controls were taken from the same hemi-diaphragms and treated as the respective test preparations except for omission of the stimulation. Fixation continued for 24 h, followed by rinsing in the same phosphate buffer, post-fixation in 1% OsO_4 in the buffer for 1 h, dehydration in acetone, and embedding in Araldite. Ultrathin sections, attempted to be kept at uniform thickness from one experiment to the other, were stained with uranyl acetate and/or lead citrate. Electron micrographs at 20,000 × and/or 40,000 × primary magnification were taken of all endplates in a section, and only one section was used from each block of tissue. All endplates thus sampled were used for collection of the numerical data. The synaptic vesicles were counted and their concentration (vesicles per μm^2) was determined within a 1800 Å wide border strip[2] free of mitochondria along the presynaptic membrane.

Stimulation experiment	Control (vesicles/μm^2)	Stimulated (vesicles/μm^2)
100 Hz, 20 sec	265 ± 15 (8)	109 ± 23 (8)[a]
50 Hz, 2 min	133 ± 15 (4)	117 ± 11 (7)
50 Hz, 5 min	178 ± 8 (9)	102 ± 18 (8)[a]
50 Hz, 5 min	215 ± 12 (14)	105 ± 10 (5)[a]
50 Hz, 10 min	193 ± 8 (8)	195 ± 14 (10)

Vesicle concentration ± standard error of mean in stimulated motor endplates as compared with controls. 5 experiments. Number of endplates examined indicated in parentheses. [a] Statistically significant reduction in the number of synaptic vesicles (t-test, $p < 0.05$).

Results and discussion. The results of our experiments are summarized in the Table. As indicated, stimulation caused a significant reduction in the number of synaptic vesicles in 3 of the experiments, whereas a non-significant reduction was found in 1 (50 Hz, 2 min) and no change at all in another (50 Hz, 10 min).

These results may appear mutually contradictory. However, it is evident from the electron micrographs that the stimulated preparations, besides containing endplates with markedly lowered vesicle number, have an admixture of endplates showing various stages of transition from the lowest to the normal vesicle concentration. Even in the 50 Hz, 10 min specimen an endplate with almost no synaptic vesicles left was found. Such large variation was never found in the control material. Thus, the population of activated endplates seems contaminated by a varying number of unactivated ones in the different experiments. A likely explanation is random alternation of endplate activation and failure of presynaptic conduction in certain fibers caused by mechanical or chemical injuries. A systematic error might then be introduced, towards the normal level of vesicle concentration. The larger dispersions present in the stimulated preparations (Table) is another aspect of this.

Representative electron micrographs from 1 experiment are displayed in Figures 1–3. Figure 1 shows a normal endplate filled with exclusively circular synaptic

vesicle profiles. Stimulated endplates (Figures 2–3) show varying degree of reduction in vesicle number. In stimulated endplates there also appears to be a definite increase in the number of elongated vesicle profiles (Figure 2). For lack of physiological evidence, the hypothesis that elongated vesicles contain an inhibitory transmitter[10] is hardly acceptable in this case. Whether the increased proportion of elongated vesicles in stimulated endplates is caused by stimulation per se, or is due to secondary artificial factors, remains unanswered.

The synaptic vesicles are claimed to be associated with a large part of the transmitter substance[11], and a reduction in the number of synaptic vesicles seems, according to the present results, to accompany neuromuscular transmission. This apparently supports the vesicle hypothesis.

As to the mechanism of transmitter release and quantitation of transmitter, some speculative remarks can be made. Many authors, to mention only COUTEAUX and PÉCOT-DECHAVASSINE[12] and NICKEL and POTTER[13], have found evidence for fusion between synaptic vesicles and the presynaptic membrane, indicating a model for transmitter release by exocytosis. The reduced number of synaptic vesicles following stimulation in the present experiments fits this concept. However, the difference in membrane composition between synaptic vesicles and the presynaptic membrane[11] makes a definite fusion between vesicles and presynaptic membrane improbable. On the other hand, if the fusion is only temporary, the present results definitely do not favour re-usage of vesicles. Release by exocytosis of complete vesicles appears unlikely because no vesicles are present in the synaptic cleft. A last theoretical possibility concerning vesicles and transmitter release is that vesicles may release the transmitter and disappear without interaction with the presynaptic membrane. This may even gain support by the fact that only a part of the transmitter is localized in association with the synaptic vesicles[11,14]. Uncertainties thus prevail as to the actual mechanism of transmitter release, and also a possible role of elongated vesicles as a stage in the release process remains hypothetical.

Zusammenfassung. Während der Fixation wurden Nervus-phrenicus-Diaphragma-Präparate der Ratte stimuliert (50–100 Hz). Die Elektronenmikroskopie motorischer Endplatten zeigte reduzierte Gesamtzahl der synaptischen Bläschen und Zunahme der Menge der «flachen» Bläschen.

H. KORNELIUSSEN, J. A. B. BARSTAD and G. LILLEHEIL

Anatomical Institute, University of Oslo, Karl Johansgt. 47, Oslo 1, and Division for Toxicology, Norwegian Defense Research Establishment, N 2007 Kjeller (Norway), 3 February 1972.

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The Hyperpolarization of Neurones of the Medulla Oblongata by Glycine

Much evidence for glycine being an inhibitory transmitter substance in the spinal cord has derived from intracellular microelectrode studies demonstrating that glycine causes a hyperpolarization of spinal neurones associated with changes in membrane conductance similar to that of postsynaptic inhibition[1–3]. There is also considerable evidence that glycine is an inhibitory transmitter in the medulla oblongata. It has been shown that glycine is a potent depressant of bulbar reticular neurones and that this depression is reversibly blocked by strychnine[4].

It has recently been reported by JOHNSTON and IVERSEN[5] that there is a high affinity uptake system for glycine in the spinal cord and the medulla oblongata. Autoradiographic studies have shown that this amino acid is actively taken up by cultured medullary neurones[6].

In the present study the action of microelectrophoretically administered glycine on the membrane potential and membrane conductance of neurones of the medulla oblongata of the cat has been investigated.

The experiments have been carried out on unanaesthetized, decerebrate cats. Decerebration was performed during halothane-nitrous oxide anaesthesia by coagulation at the midcollicular level[7]. Most animals were respiring spontaneously, but a few cats were paralyzed with intravenous gallamine triethiodide (Flaxedil) and artificially ventilated. The methods have been described in detail in a previous paper[4]. For the intracellular studies a combined microelectrode was used, consisting of a single recording micropipette (tip diameter less than 1 μm) filled with 3 *M* KCl or 2 *M* K-citrate and glued to a 4-barrel micropipette from which glycine (0.5 *M*, pH 3–3.5) was ejected microelectrophoretically. The recording electrode was fixed with Epoxylite and Deiberit 502 (dental wax) parallel to the multibarrel micropipette projecting 10–60 μm beyond its orifice[2,8]. The recording electrode was connected through an Ag-AgCl wire to a cathode follower. Potentials were displayed on an oscilloscope from which they were photographed by a Grass camera. In a few cells the membrane potential has also been recorded on a rectilinear ink recorder. The resistance of the cell membrane was measured by passing hyperpolarizing current pulses of 30–40 msec duration through the recording electrode by means of a device similar to that described by FEIN[9].

Considerable difficulties were experienced recording intracellularly from brain stem neurones. The membrane potential of the majority of cells decayed rapidly after im-

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