The action of $BaCl_2$ was changed in the same manner as was the effect of $CaCl_2$.

Papaverine antagonism in depolarized taenia coli was pronounced mainly against the contractions caused by $CaCl_2$. This inhibitory effect was seen irrespective of $CaCl_2$ addition either before or after acetylcholine action. The contractions caused by acetylcholine were comparatively less inhibited than those evoked by Ca^{++} and Ba^{++}. No inhibition was observed in the action of acetylcholine when it was added to the bathing fluid with high Ca^{++} concentration.

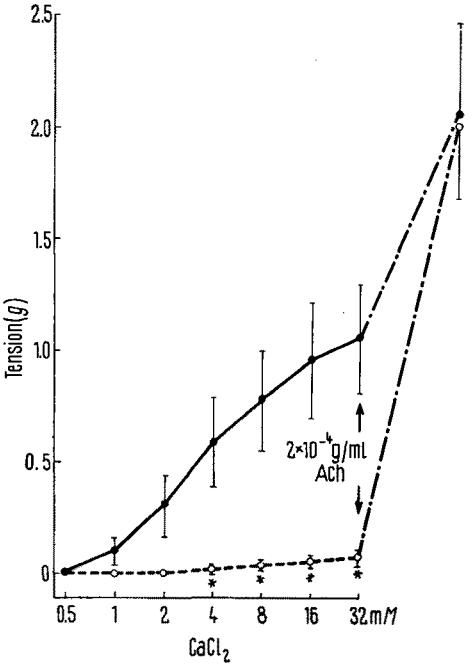

Fig. 2. Dose-response curves of $CaCl_2$ in depolarized taenia coli before and after papaverine treatment. —, controls; – – –, papaverine 1×10^{-5} g/ml; –·–·–, contractions reached after the addition of 2×10^{-4} g/ml acetylcholine. The asterisks indicate significant decrease by $p < 0.025$.

FERRARI[4] suggested that the effect of papaverine might be ascribed to an impairment of Ca^{++} availability to the contractile system. Ca^{++} available for the contractions could be derived from at least two sources. It may cross the depolarized membrane from the extracellular fluid, or it may be released from membrane store sites, for example by acetylcholine[3,5]. Our experiments provide an opportunity to distinguish between these two possibilities.

Though effective in the same dose, there was a distinct difference in the effect of papaverine in polarized and depolarized taenia coli. Papaverine in the depolarized taenia coli seemed to inhibit predominantly the flow of Ca^{++} across the membrane. In polarized taenia coli, the papaverine inhibitory action was directed predominantly against nicotine and was minimal against $BaCl_2$[1]. From our results it could be stated that papaverine influenced both nervous and muscle components of the smooth muscle preparation. The nervous component seemed to be decisive for the observed effect in polarized muscle, whereas in the depolarized taenia coli it was the interaction of papaverine with transmembrane Ca^{++} fluxes.

Zusammenfassung. Es wird die muskellähmende Wirkung von Papaverin auf die depolarisierte Taenia coli durch die Blockade des Kalziumionenstromes durch die Zellmembran erklärt.

O. KADLEC and V. BAUER

Institute of Pharmacology, Czechoslovak Academy of Sciences, Albertov 4, Praha 2 (Czechoslovakia), 11 January 1971.

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Dense-Cored Vesicles at Neuromuscular Synapses of Arthropods and Vertebrates

Electron microscopy has shown several types of vesicle in synapse-bearing nerve terminals. Often, electron-lucent 'agranular' synaptic vesicles are observed. 'Coated' vesicles, small dense-cored (granular) vesicles (about 500 Å diameter), and large dense-cored (granular) vesicles (about 1000 Å diameter), are also found in certain nerve terminals. The small dense-cored vesicles are common in adrenergic neurons and are thought to contain noradrenalin[1]. Large dense-cored vesicles are thought to contain catecholamines (eg. dopamine) in molluscs[2,3], and perhaps in other material as well[1]. The clear (agranular) synaptic vesicles probably contain acetylcholine, γ-aminobutyric acid (GABA), or other transmitter substances, depending on the neuron in which they are found [4–7]. Some neurons, e.g. those innervating molluscan muscles[8] and arthropod hearts[9,10] possess both granular and agranular vesicles.

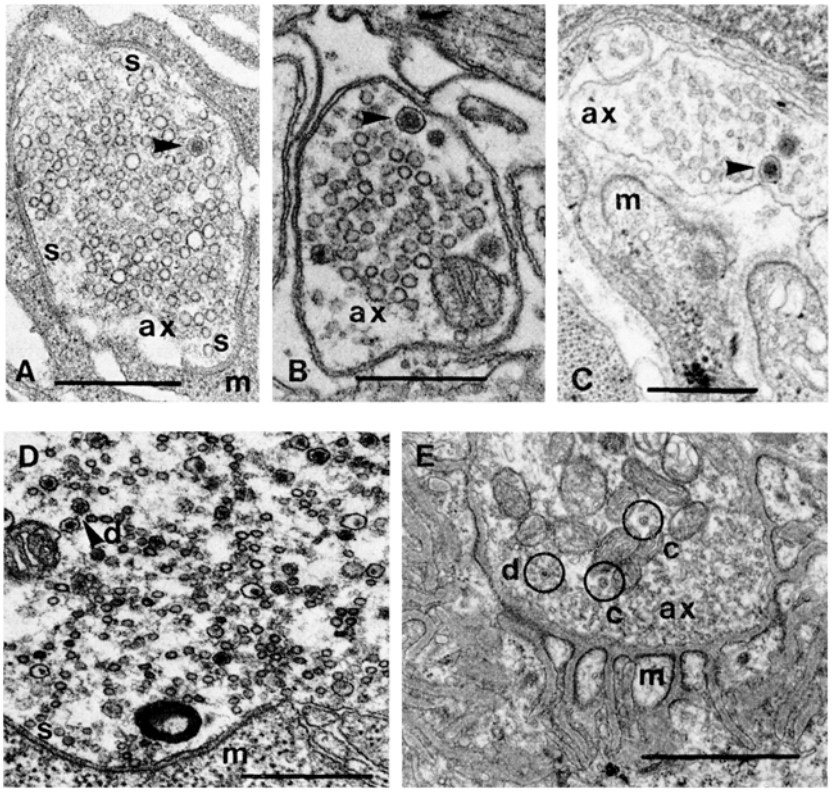
Previous descriptions of vertebrate[4,5] and arthropod[6,7] skeletal neuromuscular synapses have reported the occurrence of agranular synaptic vesicles in the presynaptic nerve terminals. In this report, we wish to show that dense-cored vesicles also occur in a wide variety of neuromuscular synapses in both vertebrates and arthropods.

Examples of arthropod neuromuscular synapses are shown in the Figure (A, B, D). These examples are from crayfish, spider and spiny lobster, respectively. In each case, both clear synaptic vesicles and dense-cored vesicles (700–1100 Å diameter) are present.

In crayfish, excitatory and inhibitory neurons can be identified by the shapes of the clear synaptic vesicles[6,7]. The transmitter agents are thought to be glutamate and GABA, respectively[6]. Both types of neuron contain similar dense-cored vesicles. Treatment with reserpine, which depletes monoamines, may deplete the dense-cored vesicles of these neurons[11].

The motor neurons of the lobster stomach muscle contained large numbers of dense-cored vesicles (Figure D). The cell bodies and ganglionic processes of these neurons are known from flourescence microscopy to contain large amounts of monoamines[12]. Electron microscopy has shown both clear and dense-cored vesicles in the same regions[13].

Dense-cored vesicles were also found at neuromuscular synapses of mammalian muscles (Figure C, E). Developing neuromuscular junctions had a higher ratio of dense-cored



Examples of dense-cored vesicles in arthropod and vertebrate presynaptic nerve terminals. Fixation was accomplished using glutaraldehyde and osmium tetroxide; staining was done with lead citrate and uranyl acetate. A) Fresh-water crayfish (*Procambarus clarkii*) leg opener muscle, excitatory axon (ax). Several synaptic contacts (s) with the muscle fiber (m) are evident. The axon terminal is filled with clear synaptic vesicles, and contains a prominent dense-cored vesicle of 800 Å diameter (arrow). B) Excitatory nerve terminal in the depressor muscle of the tarsal claw of a tarantula spider, *Eurypelma marxi*. The presynaptic axon (ax) contains many synaptic vesicles, and a dense-cored vesicle of 1050 Å diameter (arrow). C) Developing neuromuscular junction of a new-born mouse (soleus muscle). An axon (ax) in close contact with the muscle fiber (m) contains clear synaptic vesicles and a dense-cored vesicle (arrow) of 1050 Å diameter. D) Stomach muscle of the spiny lobster (*Panulirus argus*). An excitatory nerve terminal forms a synapse (s) with a muscle fiber (m). Numerous dense-cored vesicles (d) are present, in addition to clear synaptic vesicles. E) Mouse neuromuscular synapse (extensor digitorum longus, adult muscle). The axon terminal (ax) contains numerous synaptic vesicles and mitochondria. A dense-cored vesicle (d, circled) of 750 Å diameter, and two 'coated' vesicles (c, circled) appear in this terminal. Calibration marks: 0.5 μm in A, B, C, D; 1 μm in E.

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[18] Dr. Morin's address is: Biology Department, State College, Bridgewater (Massachusetts, USA).

vesicles than the neuromuscular junctions of adult animals. 'Coated' vesicles, which may be precursors to synaptic vesicles[5,14], were also observed (Figure E).

From these results, it appears that dense-cored vesicles are a common feature of arthropod and vertebrate motor and peripheral inhibitory nerve terminals. As noted above, there is some evidence from flourescence microscopy and reserpine treatments, that these vesicles may contain monoamines as in other systems[1,3,15]. The function of the dense-cored vesicles in neurons which are known to release acetylcholine, GABA, or other substances as transmitter agents is obscure. They may mediate some 'trophic' effect[16] on the muscle fibers[17].

Résumé. Les vésicules synaptiques granulées se trouvent dans les jonctions neuromusculaires de mammifères, des crustacés, et des araignées. On ne connait pas leur fonction.

H.L. Atwood, A.R. Luff
W.A. Morin[18] and R.G. Sherman

Department of Zoology,
University of Toronto,
Toronto 5 (Ontario, Canada), 11 January 1971.

Isolation of Intracellular Yolk Granules in Early Chick Embryos and Estimation of their DNA Content

Quantitative analysis of DNA in early chick embryos has demonstrated the presence of unusually high amounts of DNA in the early embryo cells during the first 18 h of development[1]. Since photometric determinations of the nuclear DNA content showed values similar to those in older embryo stages, it was concluded that the excess DNA was localized to the cytoplasm, the most likely site being the intracellular yolk granules. The latter represent ovocyte material segregated at the formation of the blastoderm and they differ markedly from the yellow granules which make up most of the yolk in the hen's egg[2-4]. A later investigation has confirmed the presence