in the anterior cell; sometimes oscillatory potentials were also observed (Figure 2b). Each GSC influenced both anterior lateral cells. It is possible that there is a less frequently encountered type of synaptic contact between these cells, something analogous, but of reverse sign, to inhibition of long duration[6]. Alternatively the linkage may not be mono-synaptic. No link has been detected among any of the different buccal cells.

From the data presented it appears that the GSC are interneurons. Certainly they receive synaptic input from many of the nerve trunks entering the cerebral ganglia[3].

The system offers great advantages for detailed studies on the mode of action of drugs which are thought to influence the levels or action of serotonin in nervous tissue and for studying the influence of two independent but identical inputs onto one cell.

Résumé. On décrit la disposition précise des contacts synaptiques réalisée par deux gigantesques neurones contenant de la sérotonine (GSC) avec d'autre neurones dans la cervelle de l'escargot *Helix pomatia*. Quelques neurones sont innervés deux fois par chaque GSC.

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Papaverine Blockade of the Calcium Action in Depolarized taenia coli of the Guinea-Pig

Recently, an antinicotinic component of the papaverine action in polarized guinea-pig taenia coli was observed. The doses of papaverine that completely blocked the action of nicotine did not depress the spasmogenic action of $BaCl_2$[1]. Conditions were looked for where also these small doses of papaverine would exert its well known myolytic action. For this purpose the interaction of acetylcholine, $CaCl_2$, $BaCl_2$ and papaverine in partly depolarized taenia coli of the guinea-pig was studied. The depolarized smooth muscle provides a means of studying the action of drugs on the contraction, under conditions uncomplicated by their effects on membrane polarization, electrical conduction or even nervous elements[2]. In this preparation the contractions could be evoked by 2 different mechanisms[3]; either by higher doses of acetylcholine, or by an increased concentration of external Ca^{++}.

Materials and methods. Taeniae coli of the guinea-pig were suspended in an organ bath in Krebs solution with 80 m*M* NaCl replaced by KCl. The spasmogens were tested before and after the addition of papaverine (1×10^{-5} g/ml). The experimental conditions concerning Ca^{++} in the bathing fluid were varied. The Ca^{++} content was 0.5 m*M* when the actions of acetylcholine or $BaCl_2$ were studied. In other experiments, no Ca^{++} but 0.5 m*M* EDTA was added to the bathing fluid. For contractions, hypertonic solutions of Ca^{++} were added. After the dissection taeniae were transferred in the bath with the depolarizing solution. In low Ca^{++} media the initial contracture disappeared within 7 min. The tension changes were measured with a mechanoelectrical transducer valve. Means $\pm$ S.E. were estimated. Each value was derived from 6 to 12 samples.

Results and discussion. The contractions of the depolarized taenia coli upon the addition of acetylcholine (3.16×10^{-6} to 1×10^{-3} g/ml) were dose-dependent. The amplitude of contractions evoked by the highest dose of acetylcholine was further augmented when the Ca^{++} concentration was increased to 16 m*M* in the presence of acetylcholine.

5 min of papaverine (1×10^{-5} g/ml) pretreatment changed the sensitivity of the preparation toward acetylcholine. The effect of low doses of acetylcholine was inhibited, whereas the effect of higher doses was decreased although not significantly. On the other hand, the effect of the added Ca^{++} in media with the highest dose of acetylcholine was significantly depressed by papaverine compared to the controls (Figure 1).

In Ca^{++}-free media the elevation of external Ca^{++} concentration led to the dose-dependent contractions in depolarized taenia coli. The addition of acetylcholine (2×10^{-4} g/ml) to the highest Ca^{++} concentration produced further contraction.

Papaverine reduced markedly and significantly the stimulatory action of Ca^{++} in the whole concentration range. In contrast, the effects caused by the addition of acetylcholine to the high Ca^{++} media were not inhibited. This contraction amplitude reached the control value (Figure 2).

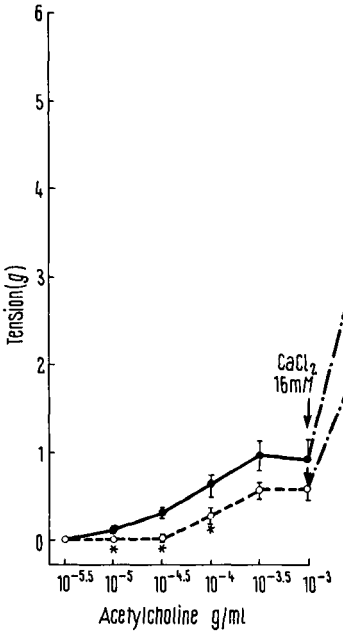

Fig. 1. Dose-response curves of acetylcholine in depolarized taenia coli before and after papaverine treatment. —, controls; – – –, papaverine 1×10^{-5} g/ml; –·–·–, the external Ca^{++} concentration was elevated from 0.5 to 16 m*M*. The asterisks indicate significant decrease by $p < 0.05$.

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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.

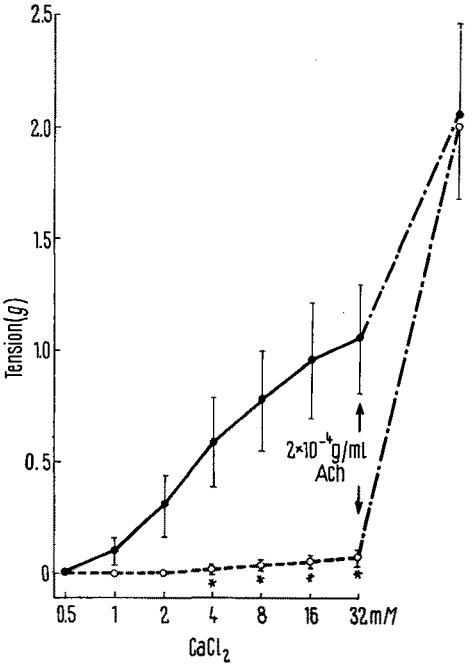

Fig. 2. Dose-response curves of $CaCl_2$ in depolarized taenia coli before and after papaverine treatment. —, controls; – – –, papaverine 1×10^{-5} g/ml; –·–·–, contractions reached after the addition of 2×10^{-4} g/ml acetylcholine. The asterisks indicate significant decrease by $p < 0.025$.

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.

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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