Pankreaskallikrein bewirkte Blutdrucksenkung durch vorherige Gabe des Hemmstoffes zu verhindern. Die Untersuchungen wurden an insgesamt 20 2,8–3,2 kg schweren Kaninchen in Urethannarkose (1,5 g/kg i.p.) durchgeführt. Die Versuchsanordnung wurde so getroffen, dass zunächst die blutdrucksenkende Wirkung von steigenden Mengen Serum- bzw. Pankreaskallikrein gemessen wurde. Anschliessend wurde APPA appliziert und danach versucht, wiederum eine Blutdrucksenkung durch Injektion von Serum- bzw. Pankreaskallikrein auszulösen. Wie aus dem in Figur 2 dargestellten Versuchsbeispiel ersichtlich ist, wird durch Injektion von 1 mg APPA/kg die durch Serumkallikrein bewirkte Hypotension verhindert. Entsprechend der Elimination von APPA (Halbwertszeit = 104 min)[5] hält die Antikallikreinwirkung bei der Gabe von 1 mg/kg bis zu 30 min und bei der Gabe von 2,5 mg/kg bis zu $2^1/_2$ Stunden nach der Injektion an. Danach verursachen auch Injektionen von Serumkallikrein wieder blutdrucksenkende Effekte. Die blutdrucksenkende Wirkung von Pankreaskallikrein wird bei Gabe der gleichen APPA-Dosis nicht signifikant beeinflusst.

Summary. The serum kallikrein-induced kinin liberation in the blood is inhibited by amidino phenyl pyruvic acid (APPA) in vitro and in vivo.

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Synaptic Connections made by two Serotonin-Containing Neurons in the Snail (*Helix pomatia*) Brain

There is a giant serotonin-containing neuron in each cerebral ganglion of the snail *Helix pomatia*[1]. This report describes the results of experiments made to locate different neurons innervated by these cells and to determine the routes of the connections. Efforts to find such cells have been centred on the buccal ganglia because previous dye injection and electrophysiological experiments[2,3] have shown that axons from the giant serotonin cells (GSCS) pass into the cerebro-buccal connectives.

Materials and methods. Electrophysiological methods were used. The circum-oesophageal nerve ring and the buccal ganglia were removed from live *H. pomatia* and pinned to a plastic sheet at the base of a small, 0.7 ml, chamber perfused with saline[4]. A double barrel glass microelectrode was inserted into one of the GSC. Similar electrodes were used to impale selected cells in each buccal ganglion. Input leads from the electrodes were fed through cathode followers to a dual beam oscilloscope or a pen recorder.

Results and discussion. Three large neurons can be seen at the lateral borders of each buccal ganglion. These cells have been called anterior, middle and posterior buccal cells. They are diagrammatically represented in Figure 1. It has been found that each of these cells receives an input from each GSC. In the case of the posterior and middle cells the input is almost certainly mono-synaptic. The input onto the anterior cells is different from that of the other cells and may involve a less frequently observed mechanism of synaptic transmission.

Spike firing in either GSC resulted in the appearance of small depolarizing potentials in both ipsilateral and contralateral posterior and middle buccal cells. The amplitude of such potentials was facilitated with repetitive firing of the GSC and there was summation which lead to spike firing. When the appropriate buccal cells were artificially hyperpolarized, the amplitude of the small potentials was increased indicating that the potentials are excitatory postsynaptic potentials (EPSPS). The latency between GSC spike and the appearance of the EPSP in the buccal cells was constant at about 100 msec. No consistent difference in latency was observed between ipsi- and contralateral connections. There was a one to one relationship between GSC spike and buccal cell EPSP even after several hundred GSCS

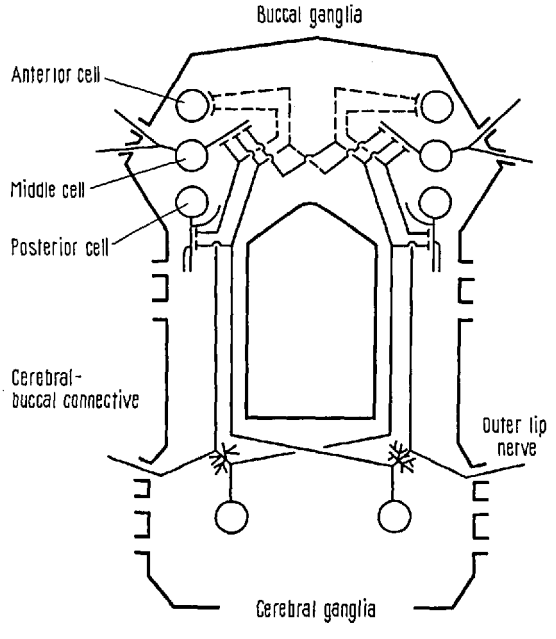

Fig. 1. Diagram showing the two GSCS in the cerebral ganglia, their processes, and the neurons with which they form synaptic contacts. The posteriorly placed cell and the middle cell in each buccal ganglion are most probably directly innervated by each GSC; the precise nature of the connections between the GSC and the anteriorly placed cell in each ganglion remains to be determined. The processes from the different cells were traced either by dye injection or by electrophysiological methods (see text). The exact points of contact between the cells is not known, but the synapses are probably axo-axonic since most if not all synapses in gastropod ganglia are of this type[6]. Note the position of the arborization of each GSC and the double innervation of the 'middle cell' in each buccal ganglion by the appropriate ipsilateral GSC.

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spikes. It appears therefore that the connections between the GSCS and each of these buccal cells are monosynaptic especially in view of the fact that reserpine, which depletes serotonin from the GSC[1] gradually reduces the amplitude of the EPSPs with repeated firing[5].

When one of the GSCs was stimulated and recordings made from either the posterior or the middle cells of the contralateral buccal ganglion, section of the ipsilateral cerebro-buccal connective had no obvious effect on the appearance of the EPSPS, whereas section of the contralateral connective abolished them. Thus the contralateral connections from each GSC are made by way of the contralateral cerebro-buccal connectives (Figure 1). Ipsilateral connections are made by the shortest route, i.e. by way of the appropriate cerebro-buccal connective. However, the middle cells also receive another link, probably monosynaptic, from the ipsilateral GSC by way of the contralateral cerebro-buccal connective and the buccal commissure. Figure 2a shows the result of an experiment in which the left GSC was stimulated and records made from the left middle lateral buccal cell. As usual, stimulation resulted in EPSPS in the buccal cell and the input increased the rate of firing of the buccal cell. After cutting the left cerebral buccal connective, EPSPS were still observed although they were smaller than before. Similar evidence has been obtained for such a link between the right GSC and the right middle buccal cell. Thus each middle cell, at least, receives two inputs from its ipsilateral GSC, one route is direct and the other runs a circular course (Figure 1). The processes leading from each posterior and middle lateral buccal cells were observed by injecting Procion Yellow (Figure 1).

The activity of the anterior cell is also influenced by the GSC, but in this case no EPSPS are observed even during pronounced hyperpolarization. GSC stimulation resulted, after a delay, in depolarization and spike firing

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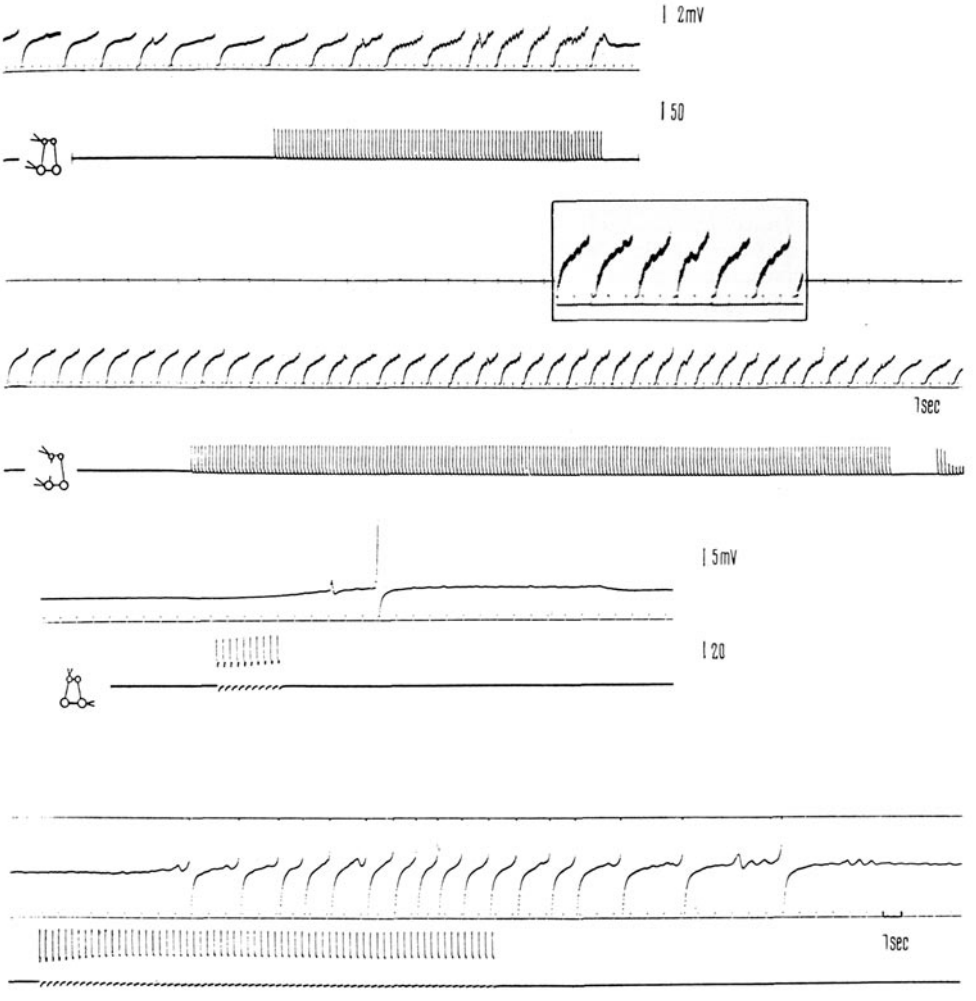

Fig. 2. a) Responses recorded in the 'middle cell' in the left buccal ganglion when the left GSC was stimulated to fire spikes, before and after sectioning the ipsilateral cerebro-buccal connective. In the top record, EPSPS are clearly observed and the rate of spike firing in the buccal cell increased. After sectioning the connective (lower record) spike firing in the GSC was less effective, but still caused an increase in activity of the buccal cell and the appearance of small EPSPS. Inset above the lower recording shows some of these small EPSPS at high magnification. b) Electrical responses of the 'anterior cell' in the left buccal ganglion to stimulation of the GSC in the right cerebral ganglion. Unlike the responses observed in the other cells, there was a delay between onset of action potential firing in the serotonin cell and increase in activity of the buccal cell. Further, the increased activity in the buccal cell persisted after the GSC had ceased firing spikes. Frequently, oscillatory potential changes were observed in these buccal cells during, or immediately after, spike activity in one or other of the GSCS.

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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Wellcome Laboratories of Pharmacology, Gatty Marine Laboratory, The University, St. Andrews (Scotland), 23 November 1970.

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[7] I thank the MRC for financial support, Dr. D. SANDEMAN for helpful suggestions and Messers J. BROWN and C. ROEMMÉLÉ for excellent technical assistance.

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).

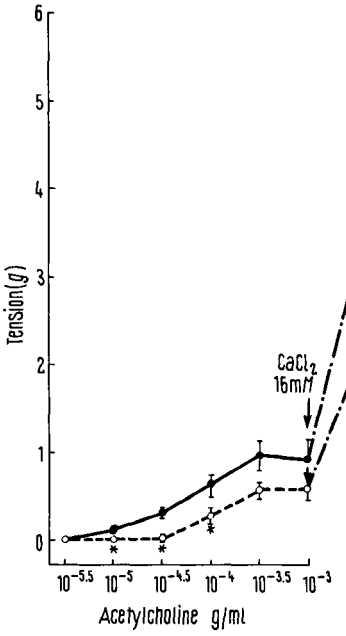

Fig. 1. Dose-response curves of acetylcholine in depolarized taenia coli before and after papaverine treatment. —, controls; – – –, papaverine 1×10^{-5} g/ml; –·–·–, the external Ca^{++} concentration was elevated from 0.5 to 16 m*M*. The asterisks indicate significant decrease by $p < 0.05$.

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