and the intermediates below fructose diphosphate increased by the addition as shown in Figure 2. This pattern is similar to that observed when incubation pH is shifted to alkaline side[4]. The actual change of the pH_i was checked by the method of CALVEY[5] using 5,5'-dimethyloxazolidine-2,4-dione (DMO). C^{14}-DMO was added to the cell suspension and the incubation was carried out as indicated above. About 0.2 unit increase of the intracellular pH was observed when citrate was added to the suspension, though the extracellular pH remained constant, being in agreement with the previous observation on ACD blood at 4°C[3].

TSUBOI and FUKUNAGA[6] observed the acceleration of red cell glycolysis by replacing Ringer solution with isotonic solutions of membrane impermeable substances. They suggested that this phenomenon is due to some unknown membrane transport function utilizing ATP. However, since no decrease of ATP by the citrate addition was observed in the present experiment, the acceleration could not be ascribed to any ATP utilization. A plausible explanation is that citrate shifts intracellular pH of red cells to alkaline side and thereby accelerates glycolysis. The present experiment may serve as an example to stress the importance of intracellular or local pH changes for regulation of metabolism.

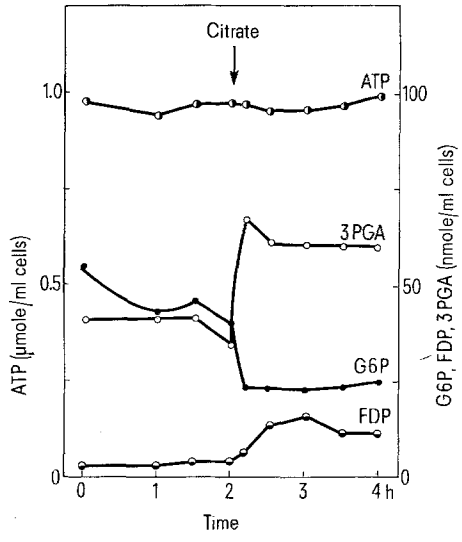

Fig. 2. Changes of glycolytic intermediates due to the addition of 33 m*M* citrate in red cells incubated at pH 7.4. Intermediates were assayed enzymatically[9]. G6P, glucose 6-phosphate; FDP, fructose 1,6-diphosphate; 3PGA, 3-phosphoglycerate.

Previously we showed that the pH_i at 4°C of red blood cells in ACD was 7.61 at the initiation of the storage and dropped to 7.12 after a month[3], indicating that the pH inside the cells of ACD blood is not so acidic as we usually expect from the observation of the pH_e at 37°C (7.0 to 6.6). It may be suggested that besides the role as an anticoagulant, citrate has another role in blood preservation media to keep the intracellular pH higher than the extracellular pH and that the use of acidified media (pH 5.0 for ACD and 5.65 for CPD) for the preservation is to keep the pH_i to physiological range, though the acidification was originally carried out to prevent caramelization of glucose during autoclaving[8].

Zusammenfassung. Nachweis, dass in Konsequenz der intra- und extrazellulären pH-Differenz, das Citrat die Erythrocyten Glycolyse beschleunigt.

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Effect of Stimulating a Central Giant Serotonin-Containing Neuron on Peripheral Muscles in the Snail *Helix pomatia*

The giant serotonin-containing neuron (GSC)[1,2] in each cerebral ganglion of *Helix pomatia* sends an axon branch into each cerebro-buccal connective, and a third axon branch into the external lip nerve[3]. The branches in the cerebro-buccal connectives form excitatory monosynaptic links with identifiable neurons in the buccal ganglia[4,5]. This report describes the results of experiments made to locate structures innervated by the axon of the GSC running in the external lip nerve.

Materials and methods. The cerebral ganglia, external lip nerves, and lips were dissected from *Helix pomatia*, and pinned to the base of a small perfusion chamber which contained 3 ml of saline[6]. A double-barrel microelectrode was inserted into one of the GSCs. The external lip nerve and its branches, and muscles located at the peripheral ends of the external lip nerves, were held in suction electrodes for stimulating and recording. Conventional electrophysiological recording methods were used.

Results and discussion. The small peripheral branches of the external lip nerve (Figure 1) contain axon branches of the GSC. The evidence is as follows: 1. Stimulation of any such nerve at its point of contact with the muscle triggered an antidromic action potential in the GSC. The antidromic action potential occurred in two steps indicating sequential invasion with an axonal potential (A spike) firing before the cell body (S spike). The axonal potential was made smaller and ultimately blocked by artificially hyperpolarizing the cell body. 2. When the GSC was stimulated directly a small spike could be recorded extracellularly from the nerves close to the lip muscles. The latency between the GSC spike and the recorded action potential was constant for any particular branch of the external lip nerve at about 30 msec., and

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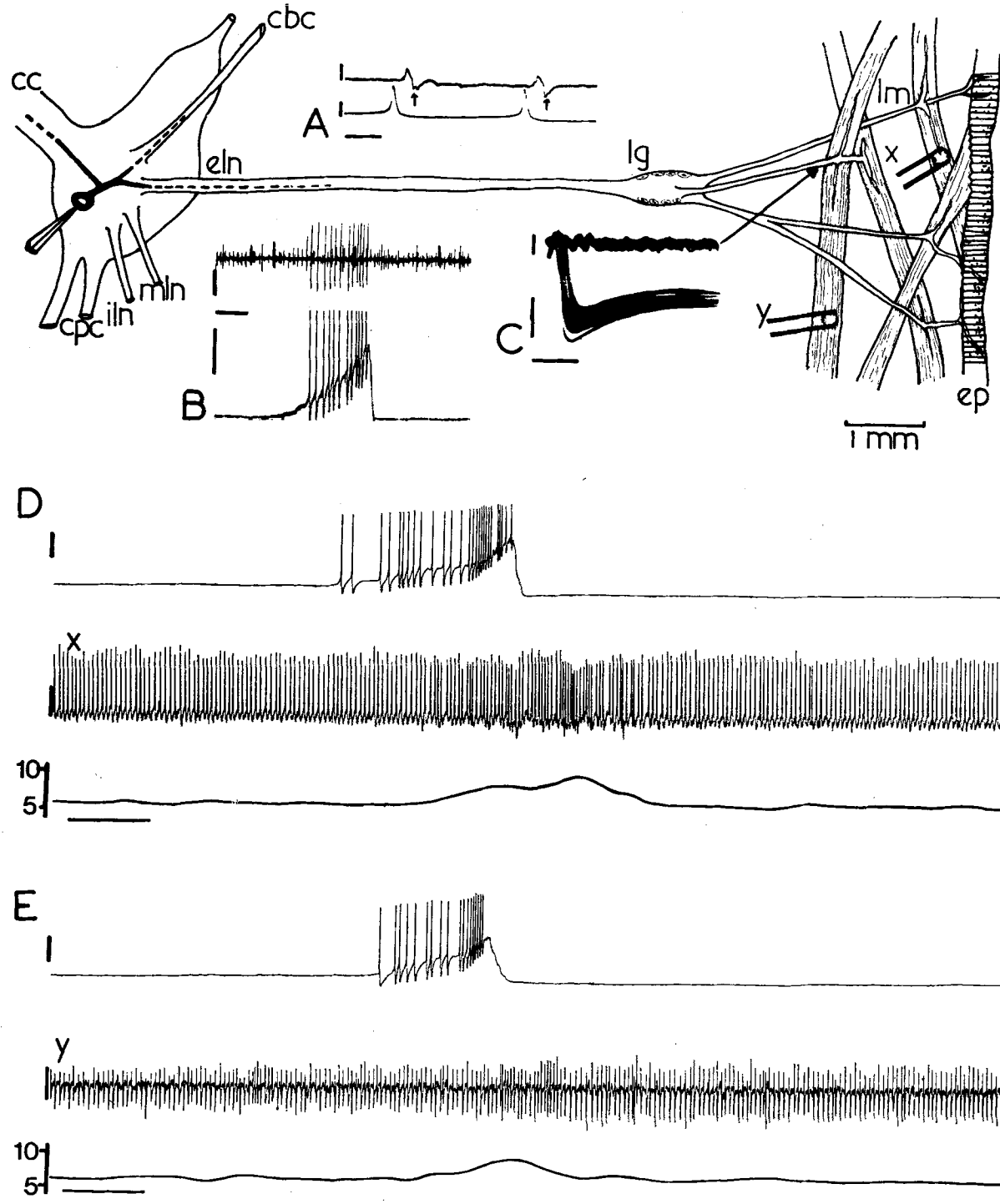

Fig. 1. The diagram (top) shows, approximately to scale, a GSC in the right cerebral ganglion of *Helix pomatia*, the nerves which contain axon branches of this neuron (eln, external lip nerve; cbc, cerebro-buccal connective; cc, cerebral commissure), and the branches of the external lip nerve which run via a small lip ganglion (lg) to the lip muscles (lm) and lip epithelium (ep). mln, median lip nerve. iln, internal lip nerve. cpc, cerebro-pedal connective. A and B) Responses recorded extracellularly in the external lip nerve half-way between the cerebral and lip ganglion (top traces), in response to GSC firing caused by a depolarizing pulse (bottom traces). A) shows that the GSC axon spike is complex in form with several inflections (arrows). The relatively constant shape of this spike indicates that more than 1 GSC axon branch occurs in the external lip nerve. Time calibration 50 msec, upper voltage calibration 120 μV, lower voltage calibration 50 mV. B) shows that the GSC axon spike is large, which suggests that one of the GSC axons is also large. Time calibration 2 sec, upper voltage calibration 100 μV, lower voltage calibration 20 mV. C) Responses recorded from a small branch of the external lip nerve (top trace) after direct stimulation of the GSC at high frequency. The small spike recorded from this particular branch (arrow) has a constant delay from the GSC action potential of 30 msec. Time calibration, 50 msec., upper voltage calibration 20 μV, lower voltage calibration 30 mV. D and E) The effect of GSC firing (top traces, voltage calibration 20 mV) on the frequency of electrical activity recorded from the lip muscles (middle and bottom traces). The arrangement and relative size of the suction electrodes used to obtain these records is indicated for example by x and y on the top diagram. The difference in shape of the muscle potentials between D (monophasic) and E (biphasic) is due to slight differences in the arrangement of the recording electrodes. Voltage calibration of middle traces is 50 μV. The lower trace in D and E is a plot of the frequency of muscle potentials per second.

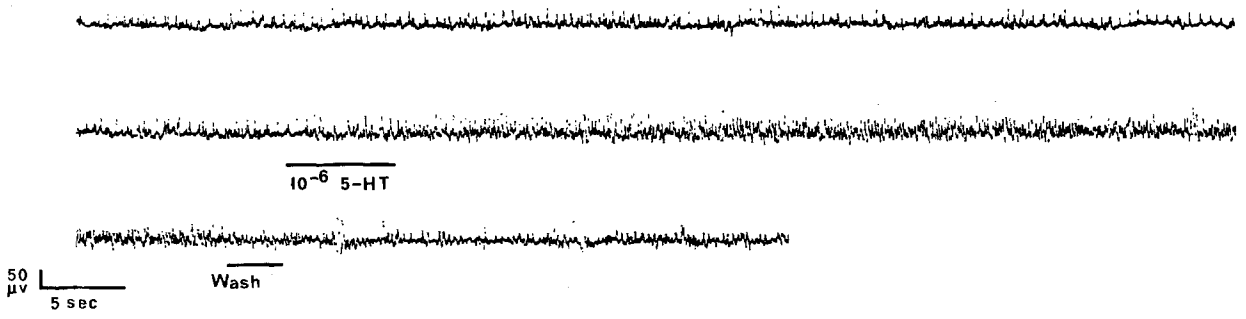

Fig. 2. Record showing the increase in muscle potential frequency caused by 5-HT (10^{-6} g/ml) applied to the muscle from the tip of a small bore pipette. The effect is reversed by washing the muscle with saline.

was the same as the latency of the antidromic potential recorded after nerve stimulation. 3. There was a one to one relationship between the GSC action potential and peripheral spike after several hundred action potentials, and after GSC firing at high frequency (Figure 1, C). The one to one relationship was not affected by bathing the preparation with saline containing high Mg^{++} and no Ca^{++}.

Individual resting lip muscles, innervated by branches of the external lip nerve, exhibited spontaneous muscle potentials which were relatively constant in frequency and amplitude. When the GSC was stimulated intracellularly to fire a burst of spikes there was a marked increase in the frequency of the muscle potentials without any noticeable change in muscle length (Figure 1, D, E). Several GSC spikes were necessary to produce this effect; no increase in electrical activity was observed after a single spike or low frequency firing from the GSC. The increase in muscle potential frequency lasted for several sec after cessation of GSC stimulation. The effect caused by GSC stimulation was abolished if the external lip nerve was severed, or if stimulating current was passed from a microelectrode placed adjacent to the GSC in the bath. The increase in electrical activity caused by GSC stimulation was mimicked by 5-HT (10^{-6} g/ml) applied locally to the muscles from the tip of a small bore pipette (Figure 2).

It is not yet clear whether the increase in electrical activity is due to 5-HT liberated from endings of the GSC directly onto the lip muscles, or whether the effect is indirect; for example via other neurons in close association with the muscles. However, fluorescence microscopy (method of FALCK[7]), bio-assay and autoradiographic experiments[8] indicate that 5-HT-containing nerve endings are present on these muscle.

The significance of the increase in electrical activity is again not yet clear, although several pieces of work appear important in relation to this phenomenon. First, TWAROG[9] has shown that 5-HT increases the occurrence of spike potentials in muscle cells of the anterior byssus retractor muscle of *Mytilus edulis* in response to nerve stimulation. Second, it has been shown by DUDEL[10] that 5-HT has an excitatory action on the crayfish neuromuscular junction by facilitating liberation of excitatory transmitter. Third, COOKE[11] has shown that exogenous 5-HT may directly facilitate neuromuscular transmission in the heart of decapod crustacea.

Finally, even if the increase in electrical activity of the lip muscles brought about by GSC stimulation is in fact indirect, it nevertheless must be considered an ultimate function of this serotonin-containing neuron.

Résumé. Le neurone géant à sérotonine (GSC) situé dans chaque ganglion métacérébral d'*Helix pomatia* envoie un axone qui se termine sur les muscles de la bouche de l'animal. La stimulation sélective du GSC provoque un accroissement significatif de l'activité électrique de ces muscles.

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Strychnine and Inhibition of Bulbar Reticular Neurones

It has been shown that strychnine, an antagonist of spinal postsynaptic inhibition[1,2], reversibly blocks the action of glycine on spinal[3,4] and on bulbar reticular neurones[5]. Furthermore biochemical[6,7], and electrophysiological investigations[3,5,8,9] strongly suggest an inhibitory transmitter role for glycine in the spinal cord and medulla oblongata. An important criterium to identify a substance as an inhibitory transmitter is the demonstration that an antagonist of the depression by the artificially administered suspected transmitter also blocks synaptically induced inhibition on the same neurone. In the present study the action of strychnine on the depression by glycine as well as on synaptic inhibition produced by peripheral nerve and cutaneous stimulation of neurones of the cat brain stem has been investigated.

Recordings were obtained from neurones of the medullary reticular formation of unanesthetized, decerebrate cats. The methods have been described previously in