Short communications

Glutamic acid mimicking of synaptic inhibition on the giant serotonin neurone of the snail

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Stimulation of the snail's tentacle nerves results in hyperpolarization and inhibition of the 'giant serotonin-containing neurone' located in the brain. Glutamic acid mimics this effect. Available data indicate that the reversal potential for the glutamic acid effect is very similar to that of the synaptic action being about 5 to 8 mV more negative than the resting potential in each case.

Introduction.—In each cerebral ganglion of the snails *Helix aspersa* and *H. pomatia* there is a large neurone located in an anterio-ventral position. Kandel & Tauc (1966) observed excitatory, but no inhibitory, synaptic potentials in these cells following stimulation of most of the different nerve trunks entering the cerebral ganglia.

Conclusive evidence has recently been obtained to show that each of these giant neurones in *Helix pomatia* contains 5-hydroxytryptamine (serotonin) (Cottrell & Osborne, 1970; Cottrell & Powell, 1971; Osborne & Cottrell, 1972) and the cells have been consequently termed the giant serotonin cells or GSCs. The 5-hydroxytryptamine within these cells probably serves a transmitter role (Cottrell, 1970a, b). It appears that the giant neurone localized in the same region of the cerebral ganglion of many different pulmonate gastropods also contains 5-hydroxytryptamine (see Osborne & Cottrell, 1972).

During the course of experiments designed to investigate the reduction in level of 5-hydroxtryptamine in each GSC following optic tentacle ablation (Osborne & Cottrell, 1972), we decided to investigate the effects of stimulating the nerves leading to the tentacles on the electrical activity of the GSC. Complex excitatory responses and also an inhibitory response were observed. This report gives a brief account of the different types of potential changes recorded, in particular the hyperpolarizing inhibitory response, and also describes the effect of glutamic acid which mimics the effect of stimulating the inhibitory nerves.

Methods. — The entire circum-oesophageal nerve ring was dissected from live *H. pomatia*. Care was taken to avoid damaging the nerves leading to the tentacles. The preparation was pinned to the plastic base of a small perfusion chamber which contained 0·8 ml of saline (Meng, 1960). Connective tissue above the GSCs was removed by careful dissection with fine tipped forceps. Frequently the GSCs could be observed through the connective tissue layer with a suitably placed light guide made from a tapered glass rod attached to a microscope lamp. All the nerves leading to the tentacles were sucked up into a tightly fitting plastic suction electrode so that they could be stimulated while still immersed in the saline. Square wave stimuli were fed from a Tektronix 160 series stimulator through an Rf isolation unit (Donaldson, 1958) to the Ag/AgCl wires arranged either side of the suction tip.

The GSC was impaled with single or double barrel electrodes. Recording electrodes were filled with either 2·6 M KCl or 0·6 M K_2SO_4 and the current passing electrodes were filled with 0·6 M Na_2SO_4 or K_2SO_4. Signals were led via a Bak cathode follower (1958), or a simple differential cathode follower containing ME 1400 valves, to a Tektronix 502A oscilloscope. Permanent recordings of electrical activity were made by recording the amplified signal from the Y plate outputs of the oscilloscope on a 220 Series Brush pen recorder. Alternatively, records were made of the unamplified signal on a Devices chart recorder. In most experiments the preparation was bathed in a continuous flow of saline.

The effects of L-glutamic acid (B.D.H.), DL-glutamic acid (Sigma), L-

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glutamic acid hydrochloride (B.D.H.) and L-glutamic acid sodium salt (B.D.H.) were tested on the cell and the same results obtained in each case. The drug solution was either simply added to the bath or applied to the cell soma from the tip (5 to 10 μ) of a broken microelectrode containing a 10^{-2}M solution of glutamic acid in snail saline.

Results.—A resting potential of about 53 to 60 mV was routinely recorded on impaling either GSC. As reported previously (Kandel & Tauc, 1966; Cottrell, 1970a), the cell is usually electrically silent although sometimes small excitatory postsynaptic potentials (SPSPs) and occasionally spikes are recorded without prior stimulation.

A burst of superthreshold stimuli applied to the tentacle nerves usually resulted in a wave of hyperpolarization in both GSCs, although it appeared that ipsilateral stimulation was more effective. The response was usually diminished with repetitive stimulation, but returned to its original level after a period of 3 or 4 min without stimulation.

The hyperpolarizing response was converted to a depolarizing one when the membrane potential of the GSC was artificially increased by applying a current (Fig. 1a). The reversal potential was relatively close to the membrane potential, i.e., about -58 to -68 mV as judged by the use of double barrel electrodes. If the GSC was made to fire spikes by artificially depolarizing the cell by about 15 mV, stimulation of the tentacle nerves resulted in cessation of action potential firing for a few seconds. Small individual inhibitory postsynaptic potentials (IPSPs) were not

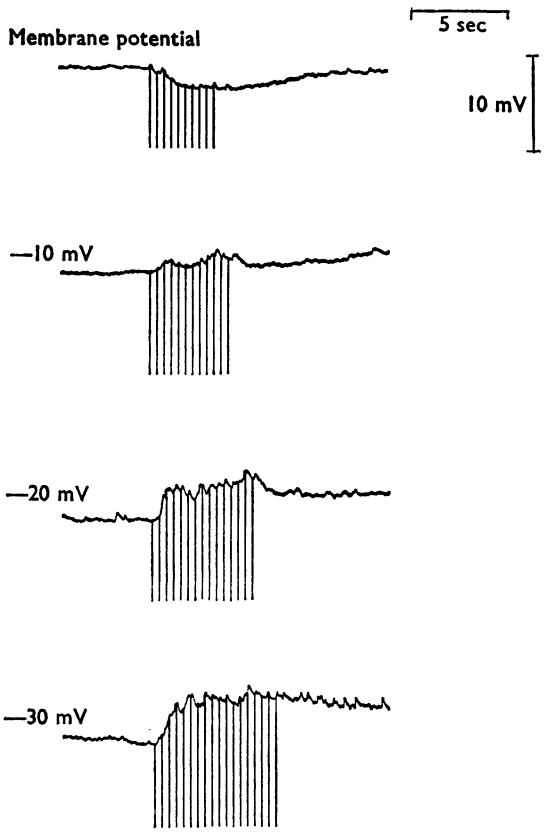

FIG. 1a. In the top record, the response of the right GSC is shown to a burst of stimuli (15 V, 10 ms duration) applied to the left tentacle nerves. The stimulus artefacts are clearly seen below the trace. In the lower records the membrane potential was artificially increased by the amount indicated by current application through the second barrel of the microelectrode and the tentacle nerves stimulated as before. As the inside of the cell was made increasingly negative the hyperpolarizing response was quickly reduced and then reversed in sign.

observed in response to the individual stimuli. The hyperpolarizing response was greatly reduced or abolished when the preparation was perfused in saline containing approximately twice the normal level of Mg^{++}, but reappeared after replacing normal saline.

Frequently, depolarizing excitatory responses were also observed when stimulating the tentacle nerves. In some cases small depolarizing potentials, presumed SPSPs, which occasionally lead to spike firing were observed before the wave of hyperpolarization; sometimes one such potential was produced by each stimulus applied even during the wave of hyperpolarization. Repeated application of bursts of stimuli frequently led to a delayed wave of depolarization with spike firing and also to delayed bursts of EPSPs, triggered presumably by some interneurones. The presence of these excitatory inputs made detailed analysis of the hyperpolarizing response more complex.

We have established that glutamic acid at a concentration of about 1 μg/ml or above in the bathing medium causes hyperpolarization and inhibition of action potentials in the GSC. The hyperpolarizing response is converted to a depolarizing response when the cell is artificially hyperpolarized (Fig. 1b). Experiments made with two separate electrodes show that the reversal potential for the glutamic acid hyperpolarization is about -64 to -70 mV. Many other active agents (acetylcholine, dopamine, noradrenaline, adrenaline, histamine, glycine and 5-hydroxytryptamine) have been tested on the GSC but they do not cause hyperpolarization or inhibition (Macon, Newton & Cottrell, unpublished observations).

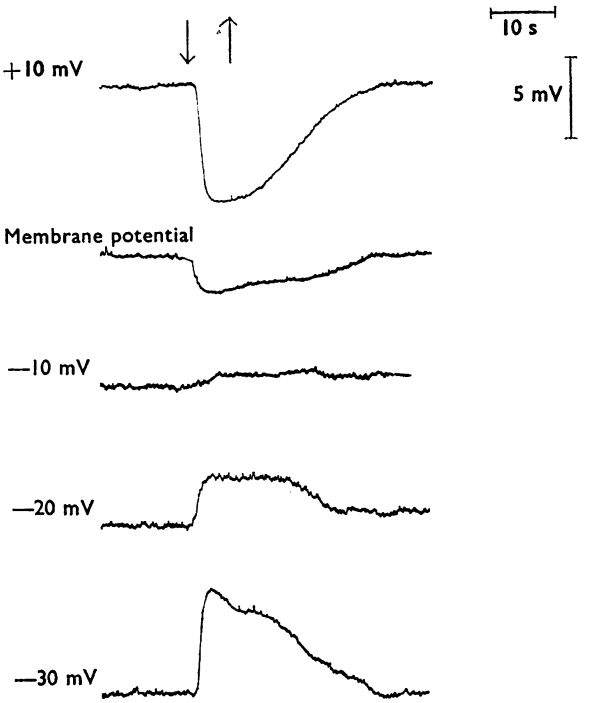

FIG. 1b. The effect of membrane potential level on the response to glutamic acid. The GSC was impaled with two separate K_2SO_4 filled electrodes and the drug was applied from the tip of a broken microelectrode filled with 10^{-2}M Na glutamate. The membrane potential of the cell was about -60 mV. The degree of artificial depolarization or hyperpolarization of the neurone from the membrane potential is indicated on the left side of the figure. At the resting potential level, glutamic acid hyperpolarized the neurone. This effect was increased if the neurone was previously depolarized, and reduced and then reversed when the cell was hyperpolarized. The level at which the response reversed sign was about 8 mV more negative than the membrane potential.

Discussion.—The results of the present study and those of Kandel & Tauc (1966) show that each GSC (i.e., each giant metacerebral neurone) received synaptic inputs from many if not all of the nerve trunks entering the cerebral ganglia. Kandel & Tauc (1966) observed EPSPs after stimulating any of the lip nerves or the cerebro-buccal, cerebro-pedal or cerebro-pleural connectives. They also noted that there were two types of EPSP which could be distinguished on the basis of their time course and discharge pattern. They did not, however, observe any inhibitory potentials. As shown in the present study depolarizing and hyperpolarizing potentials are observed after stimulating the tentacle nerves. It is therefore apparent that the activity of each GSC may be influenced by incoming synaptic activity from many different sources and it would therefore appear that these cells play an important integrating role in the cerebral ganglia.

Of particular interest to us is the existence of the inhibitory input onto each cell and the observation that glutamic acid mimics the effect of this synaptic inhibition.

Several workers consider that glutamic acid may act as an excitatory neurotransmitter in different groups of invertebrate animals (Kerkut, 1967; Kravitz, Slater, Takahasi, Bounds & Crossfeld, 1970; Usherwood, 1971). Some of the best evidence for this view comes from studies on the arthropod nerve-muscle synapse, where, for example, Takeuchi & Takeuchi (1964) and Usherwood (1969) have shown that the glutamic acid receptors are predominantly localized at the excitatory synaptic junctions.

Glutamic acid also has a potent excitatory effect on several types of neurones in the mammalian central nervous system (Curtis, 1965; Krnjevic, 1965) where it may also mediate excitatory activity between neurones.

Unlike the situation reported in these different animals where glutamic acid has an excitatory effect, glutamic acid hyperpolarizes and inhibits the GSC of the snail. This observation is in accord with the findings of Gerschenfeld & Lasansky (1964) that glutamic acid depolarizes and excites some snail (*Cryptomphallus aspersa*) neurones but hyperpolarizes and inhibits others.

In the present study, the hyperpolarizing effect of glutamic acid was reversed at a potential level of about 8 mV more negative than the membrane potential (Fig. 1b). Estimates made with double barrel electrodes of the reversal potential of the inhibitory synaptic input indicate that the neuronal response is reversed at about the same potential level which suggests that both responses are due to a change in the permeability to the same ion. However, because both excitatory as well as inhibitory inputs were stimulated and also because of possible errors due to the use of double barrel electrodes (i.e., due to coupling resistance) it is not at present possible to establish whether the glutamic acid and synaptic reversal potentials are exactly identical.

If the inhibitory input is located in one of the tentacle nerves and this nerve can be stimulated alone, it may be possible to produce synaptic inhibition without activating any excitatory input onto the GSC. This would make it easier to obtain an accurate estimate of the reversal potential of the synaptically mediated inhibitory potential, and to test further the possibility that the inhibitory synaptic activity onto the GSC is mediated by glutamic acid or some related compound.

The system described offers many advantages for a detailed analysis of the action of glutamic acid on neurones.

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