AXONAL LOCALIZATION OF AN EXCITATORY POST-SYNAPTIC POTENTIAL IN A MOLLUSCAN NEURONE

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INTRODUCTION

The excitatory post-synaptic potentials (EPSPs) of molluscan neurones presumably result, as in other cells (Fatt & Katz, 1951; Eccles, 1964), from an increase in membrane conductance to one or more ions which tends to drive the membrane toward a reversal potential near zero. Consequently, membrane hyperpolarization should increase the amplitude of an EPSP, whereas depolarization should have the opposite effect. In molluscan giant neurones, however, two factors may complicate the analysis of the conductance changes associated with the EPSP. First, the membrane conductance may be markedly voltage-dependent (anomalous rectification); consequently, hyperpolarization of the membrane may decrease the amplitude of EPSPs (Kandel & Tauc, 1966). Secondly, most molluscan synapses appear to be located on the axon and axonal branches (Gerschenfeld, 1963; Tauc, 1966), at some distance from the soma where intracellular micro-electrodes are usually inserted; thus, the analysis of EPSPs is complicated by the electrotonic spread of potential from distant regions to the soma and vice versa (Rall, 1967; Rall et al. 1967; Kehoe & Ascher, 1970).

In a preceding study of the synaptic organization of neurones in the marine mollusc, Anisodoris nobilis (Gorman, & Mirolli, 1969), we showed that EPSPs were decreased or not changed by moderate hyperpolarization and suggested that this paradoxical behaviour might be due to the anomalous conductance of the cell membrane. In this paper we show that the EPSP elicited in the gastro-oesophageal giant cell (G cell) by stimulation of the lateral nerve occurs on the cell axon at a site distant from the soma. Thus, the apparent change in conductance during the EPSP is small by comparison with changes in the cell input conductance produced by applied currents. It is the relative magnitude of the changes of membrane conductance produced by the synaptic event and by the applied currents that determine the amplitude of the EPSP.

METHODS

The majority of the electrophysiological procedures used have been described previously (Gorman & Mirolli, 1969). In brief, the gastro-oesophageal ganglion and the medial and lateral nerves of Anisodoris nobilis (MacFarland) were isolated and mounted in a chamber filled with circulating sea water maintained at a constant temperature (10–11 °C). Two independent microelectrodes filled with 3 M-KCl, one
for recording and the other for stimulating, were inserted through the intact ganglionic
connective sheath into the G cell. The output of the stimulating circuit was connected
to one of the microelectrodes through a $10^9$ or $10^8 \, \Omega$ series resistor to assure constant
current stimulation. The cut end of the medial and lateral nerves were drawn into
suction electrodes filled with sea water and each nerve was stimulated between
the suction electrode and a second electrode in the bath. Electrotonic polarization of
the G cell axon in the medial nerve was accomplished through the same electrode
system by arranging the polarity of the suction and bath electrodes (Gorman &
Mirolli, 1968).

Light and electron microscopical investigations of the G cell and its synaptic regions
were obtained from material fixed in glutaraldehyde, post-fixed in osmium and im-
bedded in Maraglas. Further details about the anatomical procedures are provided
elsewhere (Mirolli & Crayton, 1968; Mirolli & Talbott, in preparation).

Text-fig. 1. Schematic diagram of the G cell. The location of the two major synaptic regions is
indicated by knobs abutting on the cell surface. The path of lateral nerve fibres is indicated by
arrows (see text for further discussion).

RESULTS

*Synaptic regions on the G cell and the pathway of the
  presynaptic fibres of the lateral nerve*

In *Anisodoris*, as in other gastropods (Gerschenfeld, 1963; Rosenbluth, 1963;
Coggeshall, 1967), synaptic specialization, such as post-synaptic membrane thicken-
ing and subsynaptic web, are rarely seen. There is, therefore, some uncertainty in
locating synapses at the electron microscopic level. However, axonal endings con-
taining agranular (400–600 Å dia.) or granular (500–900 Å dia.) vesicles, or both, are
abundant in the neuropile of the gastro-oesophageal ganglion and in adjoining portions
of the gastro-oesophageal nerve, many of which are associated with the G cell. A
typical region of contact is shown in Plate 1. At many points of contact the extra-
cellular space between the axonal endings and the G cell membrane is markedly wider
(c. 200 Å) than observed elsewhere between adjacent axons or between axons and glial
elements (c. 100 Å). Regions with vesicles attached to the presynaptic membranes are also frequently seen. It is these points of contact which we tentatively assume to be synapses. None of these presumed synaptic structures occurs on the soma. They are seen, however, on fine branches which arise from the stem process of the soma in the gastro-oesophageal ganglion and on fine axonal branches as well as on the main axonal trunk in the nerve, 0.5 to 3 mm from the soma. The two main regions of the G cell where presumed synapses are found are illustrated in Text-fig. 1. Specialized junctions similar to those associated with electrical transmission (Bennett, Nakajima & Pappas, 1967a, b) have not been seen.

The fibres coming from the lateral nerve are typically small (1–2 μ dia.) and enter the gastro-oesophageal nerve at a distance of 2–3 mm from the ganglion. Although these fibres cannot be traced individually, bundles of them have been traced in serial sections of the lateral and gastro-oesophageal nerves and have been found to run parallel to the G cell axon in the direction of the gastro-oesophageal ganglion.

![Graph](image)

Text-fig. 2. The effects of membrane hyperpolarization on the time course of the lateral nerve EPSP. The half-width of the EPSP (width of the EPSP at half its peak amplitude) is plotted as a function of membrane potential. In this and subsequent plots the resting potential is shown at the origin of the co-ordinate axis. The inset shows a superimposition of the lateral nerve EPSP at different membrane potential levels (lower traces) with polarizing currents (upper traces).

The behaviour of the lateral nerve EPSP during soma hyperpolarization

The EPSP of the G cell produced by lateral nerve stimulation appeared in the soma after a delay of 50–100 msec and lasted 1.5–3 sec. The amplitude of the response was dependent on stimulus intensity, with cell discharge usually occurring at amplitudes in excess of 8–10 mV. The EPSP is not blocked by either d-tubocurare or atropine (A. Gorman and M. Marmor, unpublished observations) suggesting that it is not mediated by a cholinergic-type transmitter. It is, however, blocked by increasing external magnesium in the bath (see del Castillo & Engbeak, 1954) to three times
(150 mM) its normal concentration (A. Gorman and M. Marmor, unpublished observations) indicating that it is chemically mediated and not produced by electrical coupling between lateral nerve cell axons and the G cell. Except for its behaviour during membrane polarization, this EPSP resembled the typical chemically mediated EPSP encountered in other molluscan giant neurones (see Tauc, 1966). When the membrane was hyperpolarized, however, its time course was considerably reduced (Text fig. 2). In addition, its amplitude was either unaffected or occasionally slightly diminished by membrane hyperpolarization.

Text-fig. 3. Effects of changes in membrane potential on membrane conductance. The slope conductance \( \frac{dI}{dV} \) in mhos \( (\Omega^{-1}) \) measured from the dynamic current voltage relation (inset) is shown as a function of membrane potential. The resting potential \( (RP = 61 \, \text{mV}) \) is indicated by an arrow in the plot and by the zero point on the ordinate in the inset.

Both the decrease in time course and the lack of change in amplitude might be explained if the conductance of the post-synaptic membrane is voltage-dependent (Kandel & Tauc, 1966; Ozeki, Freeman & Grundfest, 1966; Nelson & Frank, 1967). Text-fig. 3 shows a plot of the G cell conductance as a function of membrane potential. The data was derived from the dynamic current-voltage curve shown in the same figure. Over a limited range of membrane potential, between 50–60 mV (resting level) and 100–120 mV, the membrane conductance increased. The EPSP could usually be examined within this polarization range: however, beyond this range, in either the hyperpolarizing or depolarizing regions, the membrane conductance increased sharply making the study of the EPSP impractical. Within this range the observed changes in EPSP amplitude with membrane polarization were, as a rule, minimal. In some cases, as shown in Text-fig. 4 A, there was a small reduction between −50 and −70 mV,
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followed by an increase in amplitude with further polarization. In other cases, the EPSP increased slightly (Text-fig. 5) or remained unchanged (Text-fig. 6A) during hyperpolarization. In those cells where a reduction occurred, it was never greater than 10–20% of the EPSP's original amplitude; conversely, the increase in amplitude at more hyperpolarized levels seldom exceeded 30% of control values. The fact that the EPSP did not decrease, nor increased slightly at high hyperpolarization levels in spite of a marked increase in total membrane conductance indicates that the EPSP is associated with a change in subsynaptic membrane conductance. However, this latter change in conductance cannot be very large since in contrast to the end-plate potential (see Fatt & Katz, 1951) the increase in EPSP amplitude is always small.

Text-fig. 4. Effects of membrane hyperpolarization on EPSP amplitude. A. Peak amplitude of the lateral nerve EPSP is plotted as a function of membrane potential. The vertical bar on the ordinate indicates plus and minus one standard deviation for the mean value of eight EPSPs measured at resting potential at regular intervals during the experiment. B. Slope conductance \( dI/dV \) of the membrane plotted as a function of membrane potential over the same range shown in A.

Direct evidence for this hypothesis was obtained in a case where the current-voltage relation of the G cell was almost linear over the whole range tested. (Cells behaving in this fashion were probably injured since their input conductance near resting levels was some three to four times larger than that normally encountered.) For this cell the amplitude of the EPSP increased at all levels of membrane hyperpolarization, and straight lines could be drawn through the experimental points for membrane potential and the peak of the EPSP at different current intensities. If these
lines are assumed to provide a reasonable approximation of membrane conductance in the presence and absence of the EPSP, then they indicate that the EPSP is associated with a 10% increase in soma-membrane conductance. The extrapolation of these lines in the depolarizing portion of the curve gives an estimated reversal potential for the EPSP of approximately +25 mV. However, if the lateral nerve EPSP does not occur at the soma membrane, then this value would be greater than the actual reversal potential at the region of the cell where it is generated, and the small change in conductance determined from soma-membrane measurements would provide a serious underestimate of the conductance change associated with the EPSP (see Ginsborg, 1967).

Text-fig. 5. Current-voltage relation of a G cell before and during synaptic stimulation. This cell had a linear current-voltage relationship (compare with Text-fig. 3). The open circles indicate the membrane potential level measured 1-2 sec after the onset of each current step. The closed circles indicate the peak amplitude of the lateral EPSP at each membrane potential level. The best fitting line through the open circles has a slope of $7.6 \times 10^{-7}$ mhos whereas that for the line through the closed circles is $8.3 \times 10^{-7}$ mhos. The inset shows superimposed current (top) and voltage (bottom) traces from the same cell with an EPSP on each voltage trace.

Effects of axonal versus somatic polarization of the lateral EPSP

Our anatomical results show that major synaptic regions occur on both the somatic branches in the gastro-oesophageal ganglion and on the axon in the gastro-oesophageal nerve. A direct way of distinguishing between these two possible locations of the lateral EPSP is to polarize the axon directly. This can be done by arranging the
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polarity of the nerve-stimulating electrodes so that a polarizing potential is electronically transmitted along the axon to the soma (Gorman & Mirolli, 1968). With this method it is possible to control portions of the axon which are electrically distant from the soma. If the lateral EPSP occurs on the axon, then it should be easier to affect its amplitude by extracellular axonal polarization, since an electrotonic potential would be considerably larger there than in the soma. Hence, its effect on the EPSP in the axon should also be greater. Conversely, if the lateral EPSP occurs on the somatic branches, then both axonal and somatic polarization should have a similar effect on its behaviour. The comparison between the two methods of polarization is shown in Text-fig. 6. The EPSP amplitude remained relatively constant during soma polarization over a potential range of \(-60\) to \(-100\) mV, in spite of a doubling of the membrane conductance (Text-fig. 6B). The behaviour of the EPSP during axonal polarization was different. Within the same soma-membrane potential range the EPSP was initially slightly reduced (< 10%) before it increased to a value approximately 50% greater than its initial amplitude. Moreover, this increase in amplitude occurred at a lower

Text-fig. 6. Effects of somatic and axonal hyperpolarization on the lateral EPSP. A, Peak amplitude of EPSP during somatic (open circles) and axonal (closed circles) polarization plotted as a function of somatic membrane potential. B, Slope conductance \((dI/dV)\) of the membrane plotted as a function of membrane potential over the same range shown in A.
membrane potential level than the slight increase produced by soma polarization. These results indicate that the lateral nerve synapses occur on the axon or axonal branches and not near the soma.

**DISCUSSION**

Our results indicate that the lateral nerve EPSP originates on the axon at some distance from the soma. The clearest evidence in favour of this conclusion is given by the results obtained with axonal polarization. This conclusion is also supported by our anatomical finding that one of the major synaptic regions of the G cell is located on the axon in the gastro-oesophageal nerve and that bundles of fibres from the lateral nerve apparently terminate in this area. Furthermore, it explains our observation that in those instances where anomalous rectification of the G cell membrane was absent, the conductance associated with the EPSP at the soma is small.

If we assume that the changes in conductance which produce the lateral EPSP are analogous to those which occur at the neuromuscular junction (see Fatt & Katz, 1951; Kandel & Tauc, 1966) then a similar change in somatic membrane conductance during the EPSP can also be demonstrated in the cases where anomalous rectification was present. The equivalent circuit for the EPSP is shown in the insert in Text-fig. 7. The membrane is represented by electromotive force $E_m$ in series with a variable conductance, $G$, whose value is a function of membrane potential, and in parallel with a fixed conductance, $g$, which is activated by a synaptic input (switch). The

![Circuit diagram for G cell.](image)
observed effects of applied constant currents, $I$, on the EPSP amplitude are explicable in terms of this circuit. The voltage, $V$, between the inside and outside is given by the equation

$$V = \frac{E_m G + I}{G + g}$$

(see Baylor & Fuortes, 1970) where $g$ is zero in the absence of a synaptic input and has a value of $g_t$ during the EPSP. It is clear from the equation that the EPSP amplitude is a function of $I, G, g_t$.

The same figure also shows a plot of EPSP amplitude versus membrane potential taken from data shown in Text-fig. 4A. The curve through the experimental points was derived from equation 1 using the slope conductance (see Text-fig. 4B) as an approximation of $G$. The curve provides a reasonable fit for the data when $g$ is assumed to be a constant and much smaller than $G$. Our data from cases where anomalous rectification was present suggest that the lateral nerve EPSP cannot be associated with more than a 10–15% change in the soma conductance of the G cell, and thus agree with the direct measurement of the synaptic conductance obtained in those cases where anomalous rectification was absent.

It should be pointed out that the agreement between the experimental data and the theoretical line can only be approximate. The region of the axon in which the lateral EPSP occurs is separated from the soma (where conductance changes are measured) by a segment of axon which, presumably, has cable-like properties. Any change in $G$ during applied currents will also change the properties of this cable. Thus, applied currents can have several complex effects on the behaviour of the EPSP. First, hyperpolarizing currents increase the soma-membrane conductance, directly affecting EPSP amplitude. Secondly, they also increase the conductance of the axon thereby reducing its length constant and increasing the electrotonic distance between an axonal EPSP and the soma compartment. Both of these effects tend to decrease the EPSP amplitude at the soma. On the other hand, hyperpolarization of the axonal membrane should increase the amplitude of the EPSP at its origin. Considering that no provision is made in the equivalent circuit for the complications introduced by the electrotonic spread of potential between different cell compartments, the fit shown in Text-fig. 7 can be taken to be satisfactory and to indicate a general agreement with the theoretical behaviour for the EPSP predicted by equation (1).

**SUMMARY**

1. Stimulation of the lateral nerve gives rise to an excitatory post-synaptic potential (EPSP) in the *Anisodoris* G cell. The amplitude of this EPSP is either unchanged or slightly reduced by moderate hyperpolarization of the soma membrane, whereas stronger hyperpolarization produces an increase.

2. Two factors contribute to this paradoxical behaviour. First, the conductance of the G cell membrane increases when it is hyperpolarized. Second, the apparent changes in cell conductance associated with the EPSP are small relative to the input conductance of the G cell.

3. There are two major regions of the G cell where presumed synaptic contacts can be demonstrated anatomically. The first is on branches of the stem process of the soma in the gastro-oesophageal ganglion neuropile, and the other is on the main trunk of the
axon and on axonal branches in the gastro-oesophageal nerve. Presynaptic fibres from the lateral nerve are present in the latter region. No synaptic contacts are found on the soma surface.

4. A comparison of the effects of axonal and somatic polarization on the behaviour of the lateral nerve EPSP indicates that the EPSP occurs on the axon or on axonal branches.

REFERENCES


EXPLANATION OF PLATE

Light (1) and electron micrographs (2 and 3) illustrating aspects of the synaptic organization of the G cell.

Fig. 1. Light micrograph of the whole axon in the proximal part of gastro-oesophageal nerve. An axonal branch (arrow) is shown arising from the axon.

Fig. 2. Medium-power electron micrograph of the G cell axon. The plane of section cuts through the origin of an axonal branch (A); other profiles (a) belonging to the same branch can be identified deeper in the neuropile of the nerve from their orientation and the characteristics of their cytoplasm.

Fig. 3. Higher-power electron micrograph of a presumed synapse between an axonal branch of the G cell (a) and a pre-synaptic fibre (p) containing agranular vesicles. Notice the widening of the extracellular space at the presumed synapse (arrow). Identified glial processes (g) are indicated.
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