TETRODOTOXIN-SENSITIVE DENDRITIC SPIKING AND CONTROL OF AXONAL FIRING IN A LOBSTER MECHANORECEPTOR NEURONE

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SUMMARY

1. A primary mechanosensory neurone, the anterior gastric receptor (AGR) associated with gastric mill muscle in the lobster foregut was examined in vitro with extra- and intra-cellular recording techniques to understand processes of dendritic integration and dendro-axonal communication.

2. AGR has a 'T'-shaped geometry; its two long (> 3 mm) primary dendrites project distally to spatially separate, stretch sensitive terminals and converge centrally onto a common apical neurite that leads to a bipolar soma and single axon.

3. The receptor's bilateral dendrites are independently capable of generating action potentials. These appear to be Na⁺ dependent since they are blocked by tetrodotoxin, but not by Co²⁺ or a lack of Ca²⁺ in the bath saline.

4. Both dendrites are autogenically active, although impulses in the dendrite with the higher intrinsic excitability may cross over and activate the trigger zone on the contralateral side. Moreover, spikes arising on either dendrite do not actively invade the soma, but are conveyed as decremented potentials to a third trigger zone on the initial axon segment.

5. Focal applications of TTX (tetrodotoxin) demonstrated the existence and allowed precise definition of a central membrane compartment of AGR that appears to lack in functional Na⁺ channels. This inexcitable region includes the soma, the apical neurite and the central branch point of the two dendrites. A failure to observe collision block of bilateral dendritic potentials as they traverse the neurite supported this conclusion.

6. Horseradish peroxidase injections and staining revealed two morphological features of the apical neurite that differed markedly from other regions of the cell. In addition to a relatively large diameter, the neurite's plasma membrane is heavily convoluted and coiled to form a lamellar transverse profile. This latter feature may itself contribute to membrane inexcitability while the former is consistent with an elevated space constant for electrotonic conduction.

7. It is concluded that the inhomogeneous distribution of membrane excitability in AGR enhances the integrative capability of the receptor's dendrites, permitting

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INTRODUCTION

The generation of action potentials in a central neurone’s axon is the emergent response to synaptic inputs arriving in the dendrites of the cell. Classically, dendritic processing of synaptic information has been considered to involve passive membrane ‘cable’ properties only, whereby input signals are transmitted in a linear manner as subthreshold polarization to a single spike initiating zone located on the neurone’s initial axon segment (Jack, Noble & Tsien, 1975). It is now clear, however, that the dendrites of certain neurones in the CNS, including cerebellar Purkinje cells (Llinás & Nicholson, 1971; Llinás & Sugimori, 1980), pyramidal cells of the hippocampus (Wong, Prince & Basbaum, 1979; Masukawa & Prince, 1984) and neocortex (Huguenard, Hamill & Prince, 1989), and certain crayfish interneurones (Calabrese & Kennedy, 1974), possess active (voltage-dependent) membrane properties that lead to spike generation and play an important role in the integrative capabilities of these cells.

At the present time, however, the functional significance of intrinsic dendritic excitability, and its contribution to the processing and transformation of synaptic information into axonal discharge remain unclear. This is due mainly to the generally small size of dendritic arborizations and their inaccessibility for electrophysiological study. Moreover, neurones in the CNS are invariably embedded in a matrix of synaptic interactions with other cells. Consequently, it is often not only difficult to differentiate between the synaptic and intrinsic origin of a given dendrite’s behaviour, but also establishing a clear functional relationship between the latter’s input/output properties remains virtually impossible.

We have begun to address these problems by using a ‘simple’ cellular model in the stomatogastric nervous system of the European lobster (Homarus gammarus). The system we study is attractive for several reasons. First, it comprises a unique identified neurone known as the anterior gastric receptor (AGR) with long bilateral dendrites which can be visualized in vitro (Simmers & Moulins, 1988a). This permits simultaneous recording of the cell’s axonal, somatic and dendritic regions and allows the latter to be isolated and/or manipulated independently with electrical stimulation, or via the focal applications of drugs. Second, AGR is a primary sensory neurone that encodes mechanical tension and movement in the muscle with which it is associated (D. Combes, J. Simmers, L. Nonnotte & M. Moulins, in preparation). Therefore, information impinging on the input terminals of the neurone can be more precisely controlled than for a central neurone which typically receives a multitude of excitatory and inhibitory synapses.

In this report we described dendritic properties of AGR which intervene in the transfer of information from the cell’s receptor terminals to its axonal membrane. We have found that the dendritic membrane of AGR is itself capable of generating action potentials at two distal sites, one along each of the receptor’s bilateral dendritic branches. We demonstrate that in contrast to most other described examples (Wong et al. 1979; Llinás & Sugimori, 1980; Masukawa & Prince, 1984) this dendritic
excitability, which is autoactive and mechanosensitive, is mediated by voltage-dependent conductances to Na\(^+\) without participation of Ca\(^{2+}\) conductances. The relationship between spiking in the two dendrites, and between the dendritic spiking and axonal discharge was also examined. Direct evidence is provided for the first time that dendro-axonal communication is mediated by a discrete intercalated compartment of inexcitable membrane, and that this regional variation in dendritic electroresponsiveness is correlated with spatial differences in morphology.

**METHODS**

Experiments (n = 45) were performed on the anterior gastric receptor (AGR) of adult European lobsters, *Homarus gammarus*. General dissection procedures were as previously described (Simmers \\& Moulins, 1988a) for isolated preparations of the stomatogastric nervous system that included the stomatogastric ganglion (STG), a section of the stomatogastric nerve (STN), the bilateral nerves containing the dendritic processes of AGR, and the stomach wall oscicle which carries the insertions of the two bundles of gastric muscle GM1 (Fig. 1.A). This combined nerve–muscle preparation was transferred to a Sylgard-lined Petri dish and superfused continuously with oxygenated artificial sea water maintained at 15–18 °C with a thermoelectric cooling system (Midland Ross Inc.). The saline composition was (mM): 400 Na\(^+\), 9·8 K\(^+\), 10·1 Ca\(^{2+}\), 52·6 Mg\(^{2+}\), 28 SO\(_4^{2-}\), 535 Cl\(^-\) and buffered to pH 7·45 with 2·5 mm NaHCO\(_3^\). In some experiments, the receptor endings of AGR in the tendon of muscle GM1 (D. Combes, J. Simmers, L. Nonnotte & M. Moulins, in preparation) remained intact and were mechanically stimulated by probing the anterior margin of the GM1 oscicle with a glass hook mounted on an electromechanical puller. In most cases, however, the fibre bundles of GM1 were cut short and AGR’s receptor endings, still attached centrally to the stomatogastric nervous system, were dissected free from the oscicle and pinned out on the Sylgard. With care, this procedure gave rise to 3–5 mm of isolated dendritic process on each side.

Extracellular recording/stimulation of AGR were made with fine platinum wire electrodes isolated electrically with Vaseline, after placement against the left and right dendritic branches and the stomatogastric nerve (STN) which carries the receptor’s axon (Fig. 1.A). In addition, the initial section of the dorsal ventricular nerve (DVN) was desheathed for access with a 3 mm KCl-filled microelectrode (10–20 MΩ) to the bipolar soma of AGR. Conventional techniques were used for display, storage and transcription of recorded data.

To examine the dependence of AGR’s action potentials on Na\(^+\) current, 10\(^{-6}\) M tetrodotoxin (TTX) was added to the saline bathing the preparation. In a subsequent refinement of this approach, the distribution of Na\(^+\) current was examined by a microperfusion technique that allowed selective application of TTX to the dendritic, somatic and axonal regions of the receptor’s membrane. This focal application was carried out with a micromanipulator-mounted micropipette positioned near (< 50 µm) a selected membrane region of AGR and containing TTX (10\(^{-5}\) M) that was ejected by low pressure (10–15 kPa) application of N\(_2\) gas via a Picospritzer (General Valves Corporation). In each trial, the roving ‘puffer’ tip was positioned upstream in the continuous flow of normal saline through the bath, and the extent of influence of the toxin was visualized and adjusted by adding dye (Fast Green) to the pipette solution. In all cases, the effects of TTX on the various membrane regions of AGR were monitored intrasomatically and by extracellular dendritic and axonal recordings.

To examine the membrane architecture of AGR, horseradish peroxidase (HRP, Sigma type VI) was pressure-injected intrasomatically for 30 min to 1 h, using a micropipette filled with the 50 mg/ml HRP in 0·2 M KCl. The enzyme was then allowed to migrate for a further 48 h at 13 °C before tissue fixation. Injection and subsequent histological procedures were as described previously (Muller \\& McMahan, 1976). Following post-fixation and dehydration, thin transverse sections of selected regions of the receptor were cut from araldite-embedded tissue and examined under transmission electron microscopy (Hitachi H600).
RESULTS

Anterior gastric receptor (AGR)

In an earlier study, Simmers & Moulins (1988a, b) identified and described a primary mechanoreceptor neurone associated with a gastric muscle of the lobster.

stomatogastric system. The bipolar cell body of this unique receptor, named anterior gastric receptor (AGR), is located in the dorsal ventricular nerve (DVN) immediately posterior to the stomatogastric ganglion (STG) on the dorsal mid-line of the stomach.
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(Fig. 1A). The soma of AGR is about 100 μm long and 35 μm in diameter and is easily visible in vitro after desheathing the dorsal ventricular nerve. The receptor's axon runs anteriorly from the cell body, through the stomatogastric ganglion and into the stomatogastric nerve wherein it projects to the bilateral commissural ganglia (not shown). There it excites two identified target elements, one in each commissural ganglion, which descend in turn to the stomatogastric ganglion via the stomatogastric nerve to excite motor neurones innervating the bilaterally symmetrical GM1 muscle of the gastric medial 'tooth' (Simmers & Moulins, 1988a). Also arising from AGR's soma is a short apical neurite which runs posteriorly for 200–500 μm in the dorsal ventricular nerve before branching into two long bilateral processes that exit the nerve to ramify eventually in the tendon of the left and right GM1 muscles (Fig. 1A; D. Combes, J. Simmers, L. Nonnotte & M. Moulins, in preparation).

Intracellular recording from the soma of AGR in isolated preparations as in Fig. 1A, reveals two important physiological characteristics of this neurone. First, the cell is invariably autoactive, firing spontaneously at low (3–15 Hz) and constant frequencies throughout the course of an experiment and in the absence of any deliberate mechanical stimulation. Second, passive stretch of either of the bilateral GM1 muscles (Fig. 1B) or active muscle contraction (not shown), causes an increase in rate of somatic impulses as a function of the amplitude of applied stretch or contraction. Our initial aims in this study, therefore, were to determine where in the
AGR its apparent autoactivity arises, and secondly, to assess the relationship between this intrinsic excitability and the transfer of mechanosensory information from the cell's distal receptor terminals to its central soma and axon.

**TTX-sensitive dendritic action potentials**

Because of the spatial separation (> 6 mm) of the bilateral receptor fields of AGR, its primary dendritic processes can be independently recorded/stimulated with extracellular electrodes, along with a microelectrode placed in the soma (Fig. 2A). In such experiments, two types of depolarizing transient associated 1:1 with impulses occurring spontaneously on either dendritic branch can be observed in the cell body of AGR. These intrasomatic potentials, which are always preceded by a corresponding dendritic impulse, are never more than 20 mV in amplitude. In the preparation of Fig. 2A, for example, spikes occurring spontaneously in the left dendrite gave rise to 10 mV potentials in the cell body, while impulses on the right dendrite were followed by 20 mV soma potentials. The first conclusion from these observations, therefore, is that the two primary dendrites of AGR are each capable of generating action potentials. Moreover, the temporal relationship of this spontaneous activity with that observed in the soma suggests that the receptor's autoactive capability resides in the spiking mechanism of the dendrites themselves. This is confirmed in the experiment of Fig. 2B, where autogenic tonic firing was observed (throughout a recording time of 1 h) in a dendritic process of AGR after it had been physically disconnected from the remainder of the cell.

Given the sensory function of AGR, what is the relationship between this intrinsic dendritic activity and extrinsic input to the neurone? Figure 3A shows spontaneous spiking in an isolated dendritic process of AGR, but in this case with its peripheral projections to the GM1 muscle remaining intact. Gentle pressure exerted on the muscle attachment, or passive stretch of the muscle itself, evoked an increase in frequency of ongoing dendritic activity which was dependent on the strength and duration of the mechanical stimulation. Evidently therefore, the spike-generating mechanism of AGR's dendrites is able to encode mechanical input, providing an active (frequency modulated) carrier signal for information transfer from the site of mechanotransduction in distant receptor terminals towards the cell's soma and central axon.

Since the dendrites of AGR clearly have the ability to produce action potentials both in an autogenic and mechanodependent manner, we were interested to determine the ionic mechanism responsible for this membrane excitability. As illustrated in Fig. 3B (also see Fig. 7), a fast sodium conductance appears to be involved, since the addition of tetrodotoxin (10⁻⁶ M) to the bath saline suppressed all spontaneous dendritic (and somatic) activity, and the cell no longer responded to strong electrical or mechanical stimulation of either dendrite. By contrast, the removal of Ca²⁺ ions from the saline, or the introduction of Co²⁺ ions, did not block the spike generating capability of AGR's dendrites (not illustrated). Therefore, unlike central neurones in which Ca²⁺-mediated dendritic spikes predominate (see Discussion), action potentials in the dendrites of AGR appear to be produced by a membrane conductance to sodium.
**Dendro-dendritic interactions**

In the experiments of Figs 2A and 3B, the left and right dendrites of AGR appear to be operating as independent entities, their different inherent rates of firing giving rise to temporally unrelated events in the receptor's soma. In most preparations (> 70%), however, the two dendrites were strictly co-ordinated in their activity patterns while in 10% of preparations bilateral co-ordination was seen to vary spontaneously during the course of an experiment. An example of this flexibility is illustrated in Fig. 4A, where impulses in the two dendrites occasionally arose independently of each other, while at other times (see arrowheads) they appeared to fire in phase-coupled pairs. This tendency towards bilateral coupling is further evident in Fig. 4B from the same experiment, which shows the interval distribution of spontaneous spikes arising alternatively in the two dendrites of the cell. Although latencies between bilateral spike pairs varied over a 300 ms range, on most occasions (> 60%) the two dendrites fired within 60–120 ms of each other, indicating a direct interaction between the spike trigger zones of the two sides. In addition, this dendro-dendritic interaction was readily influenced by the injection of low levels of tonic
current into the soma of AGR (Fig. 4C). With depolarizing current (+0.5 nA), the two sides were locked in strict co-ordination (left panel, Fig. 4C): the impulse on the right dendrite always preceded that of the left side (cf. Fig. 4A), with each spike contributing to an appropriately timed potential doublet in the receptor’s cell body.

Fig. 4. Bilateral co-ordination of dendritic spiking. A, autogenic activity recorded intrasomatically (at resting potential) and extracellularly from the left and right dendrites of AGR (see schema). Arrowheads indicate soma potential doublets arising from the close succession of impulses in the right and left dendrites, respectively. B, distribution of latencies between successive pairs of bilateral dendritic spikes measured from records as in A. In most cases the left dendrite fired from 60–120 ms after its contralateral partner. C, injection of tonic depolarizing current (+0.5 nA) into the cell’s soma (left panel) produced 100% coupling between the two sides (cf. A), while with tonic hyperpolarization (−0.5 nA, right panel) the dendrites fired at unrelated frequencies. Note that a change in size of the intrasomatic potentials was not evident at these small current levels. D, superimposed oscilloscope sweeps (5) of dendritic activity in a different preparation: a, spontaneous impulses on the left (L) dendrite are followed 1:1 and at constant latency by right (R) dendritic impulses; b, electrical stimulation of either dendrite evokes impulses on the contralateral side.

In contrast, hyperpolarizing current (−0.5 nA) completely decoupled the two sides (right panel, Fig. 4C); although both dendrites remained autoactive, they now fired at different frequencies and consequently produced unco-ordinated soma responses. It is important to note that the less active dendrite in the coupled pattern of Fig. 4C (right panel) is the follower side during coupled activity at resting potential (Fig. 4A) or during soma depolarization (Fig. 4C, left panel). Under these experimentally
imposed conditions therefore, the retrograde spread of injected current from the soma is able to facilitate or block bilateral dendritic interaction, presumably by altering the safety factor for transmission through the medial branch point with the apical neurite to the opposite side (see below).

Moreover, each dendrite has equivalent access to the spike trigger zone of its contralateral partner, as seen in the dendritic recordings from a different preparation in Fig. 4D. In this example, autogenic activity in the two dendrites remained in strict co-ordination throughout the experiment, with the leading spike in each bilateral pair originating on the left side and followed at constant latency by a right dendritic spike (Fig. 4Da). However, impulses evoked by electrical stimulation of either the trailing (right) or leading (left) dendrite in the spontaneous pattern triggered 1:1 spikes on the contralateral side (Fig. 4Db). Thus, although both dendrites are autogenically active, it appears that the dendrite with the higher intrinsic excitability tends to impose its own pattern, each impulse crossing-over and activating the trigger zone of the opposite partner (Fig. 4A, B and Da). When cross-excitation fails to occur, either spontaneously (Fig. 4A, see also Figs 2 and 3Bb) or in response to experimental hyperpolarization (Fig. 4C), the two sides effectively behave as functionally independent compartments of the same neurone. The following section examines the relationship between these dendritic compartments and the receptor’s output axon.

**Dendritic control of axonal firing**

A first step to understanding the nature of dendro-axonal communication in AGR was to monitor spontaneous activity in different regions of the cell including its axon, again using extra- and intra-cellular recordings. As seen in Fig. 5, a third type of transmembrane event can be observed in the cell body of AGR, in addition to potentials of dendritic origin described above. These large somatic potentials are
unlike the smaller dendritic potentials in that they are correlated 1:1 with impulses recorded along the cell’s axon. On the basis of their timing and amplitude, we conclude that these large intrasomatic transients are reflected axon spikes arising at a trigger zone in the initial axon segment, electrically close to the rostral margin of

![Diagram of neuron connections](image)

Fig. 6. Triggering of axonal spikes by dendritic action potentials. Superimposed oscilloscope records showing somatic and axonal responses (recorded in the stomatogastric nerve, STN) to electrical stimulation of one (A) or both (B) dendrites of AGR (see diagram). A, soma potentials evoked alone by stimulation of the right dendrite (a), generate axonal spikes (b) when the cell body is slightly depolarized with injected current. B, decreasing the interval between paired stimulation of the two dendrites (S_R - S_L) produces soma potentials (a) that eventually sum to trigger axonal action potentials (b). Arrows above extracellular traces indicate stimulus artifacts.

the receptor’s bipolar soma. The relationship between these axonal impulses and spikes of dendritic origin seen during spontaneous activity as in Fig. 5, is illustrated in the faster oscilloscope records of Fig. 6 where direct electrical stimulation was used to elicit dendritic firing. Firstly, impulses arising distally on one or other dendrite do not actively invade the axon of AGR, but arrive as subthreshold depolarization. In Fig. 6.Aa, for example, single brief shocks to the right dendrite evoked soma potentials identical to those produced by spontaneous dendritic impulses (not shown), but without any response in the cell’s axon. However, when the soma
Demonstration of was placed proximal gives now in a bilateral excitable versus potentials arising potentials; action of the temporal brief, temporal summation occurred and threshold for axonal spike was now reached (Fig. 6B).

In terms of controlling axonal firing therefore, dendritic impulses appear to behave in a manner analogous to conventional postsynaptic potentials: as each dendritic spike propagates centripetally, at some point it seems to fail and further transmission through the soma and beyond to the axon trigger zone is via electrotonic conduction. If attenuation at this time is sufficient, the depolarizing wave fails to cross the axonal threshold and no spike will occur. On this basis, however, and like EPSPs, dendritic potentials arising in different distal regions of AGR can summate centrally and trigger an axonal action potential whereas either alone may not.

Demonstration of an inexcitable compartment

Where precisely is the presumed passive membrane located in AGR and what is the extent of its distribution? Although the somata of most arthropod neurones are incapable of supporting action potentials, as demonstrated in the following experiments, AGR possesses an inexcitable region that extends far beyond its soma boundary, projecting caudally along the apical neurite at least until the branch point of the two main dendrites.

The first strategy used was to take advantage of the dependence of AGR’s dendritic (see Fig. 3B) and axonal (see below) action potentials on fast Na⁺ channels. By selectively perfusing different regions of AGR with TTX using a ‘puffer’ micropipette (see Methods), we were able to assess the spatial distribution of excitable versus inexcitable membrane and the latter’s contribution to dendro-axonal communication. An example of one such experiment, in which the receptor’s bilateral dendrites, soma and axon were monitored simultaneously, is shown in Fig. 7. Important to note here are the locations of the two dendrite recording electrodes (Fig. 7A–E); the one on the left dendrite (L) being positioned relatively close to the proximal branch point with the apical neurite, while that on the right side (R) was placed considerably more distal, some 3 mm from the branch point.

Under normal saline (Fig. 7Ab), spontaneous activity typical of AGR in vitro was observed; action potentials originating on either dendrite followed 1:1 by intrasomatic potentials which occasionally were able to summate and trigger an axonal action potential. Focal application of saline (<10 μl) containing TTX (10⁻⁵ M) from the ‘puffer’ pipette directed at the left dendrite (L) on the distal side of the recording electrode (Fig. 7Ba) rapidly (within seconds) led to the suppression of impulses in the branch itself (Fig. 7Bb; upper trace) and consequently, a loss of
Fig. 7. Regional variations in sensitivity to TTX. Panel a, placement of recording electrodes (left and right dendrites, axon and soma) during focal application of TTX ($10^{-6}$ M) to different regions of the same receptor. Panel b, corresponding responses, in each case followed by recovery in normal saline. A, in the absence of TTX, spikes arising spontaneously in both dendrites are recorded in the soma and occasionally trigger axonal action potentials. B, TTX applied distally to the left dendrite (note more proximal recording position) abolishes spikes in this process but not in the contralateral dendrite. C, TTX applied proximally to the right dendrite (note more distal recording position) fails to suppress peripheral generation of spikes (see second trace), but blocks their access to
corresponding soma potentials (third trace). By contrast, action potentials continued to arise spontaneously on the contralateral (non TTX-perfused) dendrite (R, second trace) and manifest themselves in the cell body. Note that prior to TTX application, control ejections of normal saline plus dye (see Methods) from the same micropipette positioned at similar or even shorter distances from AGR’s dendrite had verified that the carrier alone was without effect. In this experiment the field of influence of the microperfused TTX at the receptor’s membrane was around 100 μm, equivalent to the length of AGR’s soma.

Following recovery of control levels of activity in the TTX-treated dendrite (recovery times were 20–30 min with normal saline circulating in the bath throughout the experiment), the toxin-containing pipette was transferred to the right dendrite and positioned on the proximal side of the recording electrode, within 200 μm of the medial branch point (Fig. 7Ca). In this configuration, focally applied TTX had no effect on impulse generation in the distal region of this dendrite (Fig. 7Cb, second trace), but transmission of its spikes to the soma was virtually abolished (third trace). This observation not only points further to a peripheral site for autogenic firing in the dendrites of AGR, but also indicates that the dendritic membrane is excitable until within 200–300 μm of the branch point with the apical neurite; when Na+ channels are blocked in this region, action potentials propagating centripetally from a more distal locus fail to attain the soma and the two compartments are effectively decoupled. By contrast, impulses generated on the contralateral dendrite (Fig. 7Cb, upper trace) which was now unexposed to the toxin (cf. Fig. 7B), continued to be conducted to the soma with similar amplitudes as seen under control conditions (cf. Fig. 7A).

The third region of AGR tested with TTX microperfusion was the membrane of the apical neurite between the cell body and the branch point of the two dendritic processes (Fig. 7Da). Unlike its effects on dendritic membrane, the presence of TTX at all points along this region and including the soma, had no observable effect on dendro-axonal communication (Fig. 7Db); even with repeated applications of toxin, action potentials occurring spontaneously in either dendrite (top two traces) gave rise to soma potentials that were similar in shape and amplitude to those observed when TTX was absent (cf. Fig. 7Ab). As under control conditions, moreover, the arrival of appropriately timed potentials from the two sides were able to summate and reach threshold for an impulse that propagated up the cell’s axon (lower trace). By contrast, the ejection of TTX onto the initial segment of AGR’s axon (Fig. 7Ea), some 100–200 μm rostral to its cell body, suppressed the production of these impulses (Fig. 7Eb; bottom two traces), but without affecting ongoing dendritic spiking or the transmission of this activity to the soma.

From these regional differences in sensitivity to TTX we conclude that, unlike AGR’s axon and two dendritic branches, the membrane of the apical neurite and soma is lacking in functional Na+ channels. Under normal circumstances, therefore,
a dendritic action potential arriving at the distal boundary of this inexcitable compartment fails, and further somatopetal conduction is electrotonic, the depolarizing wave spreading decrementally along the passive neurite and cell body until the initial axon segment.

Fig. 8. Lack of collision between bilateral dendritic action potentials. Oscilloscope records show somatic responses (with delay $D_L$ and $D_R$, respectively) to dendritic action potentials evoked by paired electrical stimulation of the left ($S_L$) and right ($S_R$) dendrites (see diagram). With a decreasing interval between stimulus pairs ($A-C$), the soma responses gradually summed until threshold for an axonal spike is reached ($D$ and $E$). Note the simultaneous arrival at the soma of the bilateral dendritic potentials in $E$. With a further decrease in stimulus interval ($F$ and $G$), the arrival of the right dendritic potential can be seen to precede the left side, and depolarization is not insufficient for axonal spike generation. This transmission of dendritic impulses at all times is consistent with membrane inexcitability throughout the entire apical neurite.

Moreover, given the ability of AGR’s two main dendrites to produce action potentials independently, it is reasonable to assume that this central inexcitable compartment extends beyond the point of confluence with the apical neurite and into the initial dendritic segments themselves. The functional implications of this are illustrated in the soma recording of Fig. 8 where electrical stimulation of the two dendrites was timed to produce action potentials arriving in close succession at the
apical branch point. In this experiment we reasoned that the presence of active membrane at the branch point or more proximally along the neurite itself, would be revealed due to relative refractoriness following a dendritic action potential causing complete blockade of a closely succeeding one. Figure 8A shows the somatic responses to dendritic action potentials resulting from successive stimulation of the left (S_L) and right (S_R) dendrites, respectively. As the interval between the paired stimuli was gradually decreased (Fig. 8B and C), the second dendritic potential remained apparent and eventually summed with the first depolarizing transient to trigger an axonal action potential (Fig. 8D). In Fig. 8E, the stimulus interval was adjusted for the simultaneous arrival of the two dendritic impulses at the branch point (and hence at the soma); evidence for spike failure resulting from collision was still not observed, and even after a complete reversal in timing of stimuli so that the right dendritic spike now preceded that originating from the left side (Fig. 8F and G).

In conclusion, therefore, membrane unable to support action potentials appears to be uniformly distributed along the entire length of AGR’s apical neurite and probably beyond, into the proximal segment of each dendritic process. Although the geometry of the neurone at the branch point may itself contribute to propagation failure (see Discussion), with this configuration of membrane inexcitability, information arising separately on the two dendrites is at all times permitted access, albeit with attenuation, to the soma and axon. Noteworthy finally is the difference in amplitudes of soma potentials generated by dendritic spikes of the two sides (Fig. 8; see also Figs 2A, 3B and 5). Presumably this reflects bilateral asymmetries in effective electrotonic distance over which impulses originating in the two processes must cross.

Structural correlates of membrane properties

In light of the physiological properties of AGR described above, we were interested to see whether morphological properties revealed by intrasomatic injection of horseradish peroxidase (HRP) provided further clues about the receptor’s electrical behaviour. In particular, we sought regional variations in fibre diameter or other structural differences that may contribute to the non-propagation of impulses in the cell’s apical neurite.

Transverse sections of AGR’s axon (at ca 500 μm from the soma boundary), a dendrite (ca 1 mm from its point of bifurcation) and primary neurite (midway between branch point and soma) from the same preparation following HRP injection are illustrated in Fig. 9A–C, respectively. At each level AGR is enwrapped by a multilayered envelope of unmarked glial cells. However, two important regional differences in AGR’s structure are evident in the profiles of Fig. 9. First, the apical neurite is considerably wider than either the axon or peripheral dendrite, both of which are typically only 3–8 μm in diameter (Fig. 9A and B), while the neurite can reach a diameter of 30 μm or more (Fig. 9C). Second, whereas the axonal and dendritic profiles are characterized by smooth perineuronal contours (Fig. 9A and B), the membrane of the apical neurite is strikingly complex, displaying extensive invaginations and protrusions that in places may completely re-envelope themselves (Fig. 9C). Closer inspection of these lamellar regions reveals peroxidase staining of the cytoplasm on either side of plasma membranes lying in close apposition (Fig.
Fig. 9. Ultrastructural differences between AGR’s axon (A), excitable dendrite (B) and inexcitable apical neurite (C and D) seen in transverse sections of an HRP-injected neurone. Both the axon and dendrite (A and B) have a considerably smaller diameter than the neurite (C), and the latter’s membrane perimeter exhibits peculiar infoldings and
9D), thus confirming that these bilateral profiles were continuous projections of the same cell, namely AGR, rather than a glial-neuronal boundary.

Similar regional differences in diameter and membrane architecture were observed in all four AGR neurones that we injected with HRP. Although some distortion resulting from tissue shrinkage during histological processing undoubtedly occurred in these preparations, we do not see how this could have led preferentially to the unusual transverse morphology of AGR’s apical neurite as seen in Fig. 9. We conclude, therefore, that these are structural features of the mechanoreceptor with potentially important consequences for the transmission of electrical information.

DISCUSSION

In the present study, we have taken advantage of the large geometry and accessibility of an identified stomatogastric neurone, the anterior gastric receptor (AGR; Fig. 1), to examine its physiology and structure. The division of this unique cell’s dendritic arbor into two long and spatially separate regions that are easily monitored in vitro along with the neurone’s soma and axon provides a convenient model for examining membrane excitability within, and communication between, these different cellular compartments. Moreover, that this peripheral neurone is a primary mechanoreceptor cell enabled us to study the electroresponsiveness of its dendritic membrane without interference from the many synaptically driven conductances which characterize neurones of the central nervous system. The major findings of our study are summarized in Fig. 10.

**Autogenic Na⁺-dependent dendritic spiking**

As reported previously (Simmers & Moulins, 1988a), a feature of AGR’s behaviour in both minimally dissected *Homarus* and completely isolated preparations, is an apparent autoactive spiking capability consisting of continuous tonic firing at constant frequency. Indeed, in these earlier experiments and those reported here, AGR was never observed silent even in the absence of any detectable mechanical tension placed on its dendritic terminals. Our present experiments demonstrate that this autogenic firing is due to an intrinsic property of the receptor’s dendrites themselves. Firstly, in multiple extracellular recordings, spontaneous impulses occurring along one or other bilateral process are always seen to precede, rather than follow, somatic responses (Figs 2–4) which in turn precede any action potentials propagating up the receptor’s axon (Fig. 5–8). Secondly, when isolated from each other and the rest of the stomatogastric nervous system, both dendritic processes continue to fire spontaneously at their inherent frequencies (Figs 2B and 3A).

Autoactive dendritic impulses have been reported in a variety of neurones in the CNS including cells of the mammalian cerebellum (e.g. Llinás & Sugimori, 1980) and hippocampus (e.g. Wong et al. 1979). However, a fundamental difference between

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 coilings. D, magnification of this peripheral region showing two lamellar expansions of AGR’s principal intraneuritic space (N). Arrows indicate the close apposition of plasma membrane resulting from this wrapping.
these examples and AGR is that, on the basis of their sensitivity to TTX, dendritic action potentials in the latter appear to be generated by sodium rather than calcium channels (Figs 3B and 7). Claims of dendritic spiking produced by Na⁺ are rare (Jefferys, 1979), being only demonstrated in cell types that are also known to have predominantly Ca²⁺-dependent dendritic impulses (Schwartzkroin & Slawsky, 1977; Wong et al. 1979; Masukawa & Prince, 1984) or have been subjected to pathological influences such as axotomy (Sernagor, Yarom & Werman, 1986) or cell dissociation procedures (Huguenard et al. 1989). To our knowledge, the dendritic spiking described here is the first reported example of a Na⁺-dependent mechanism demonstrated in an intact neurone, and in which peripheral impulses are involved in orthodromic communication (cf. Jefferys, 1979) with the cell's central axon.

The functional significance of autogenic excitability in the dendrites of a primary mechanoreceptor neurone remains a matter of speculation although clearly this intrinsic behaviour is accessed by mechanotransduction processes occurring in the cell’s receptor terminals (see Fig. 3A). Therefore, in a manner equivalent to synaptic modulation of autogenic Ca²⁺ spikes in the dendrites of certain central neurones (see Wong et al. 1979; Masukawa & Prince, 1984), a likely role of AGR’s autoactivity is
to increase the cell’s sensitivity to extrinsic (mechanical) input which is immediately encoded by ongoing intrinsic activity of the dendrite (rather than first having to attain threshold for firing), then conveyed as a digital signal to the receptor’s distant (3–6 mm) central axon.

*Multiple sites for spike initiation and dendritic integration*

In most cases where dendritic electroresponsiveness has been demonstrated, it has been presumed to be associated with a non-uniform distribution of membrane properties whereby regions of excitable membrane (‘hot spots’) along the dendritic tree are separated by portions of membrane unable to support action potentials (Llinás & Nicholson, 1971; Calabrese & Kennedy, 1974; Traub & Llinás, 1979; Wong et al. 1979; Llinás & Sugimori, 1980). Indeed this is formally the reason for the existence of separate spike-initiating sites in different topographical regions of the same neurone. In these circumstances, when dendritic spikes do not actively invade the soma, they may serve a similar function to conventional PSPs, contributing only subthreshold excitation to a main axonal spike-initiating zone.

In the present study, we find direct evidence for the existence of a region of inexcitable membrane along AGR’s soma neurite until, and including, the point of bifurcation to the left and right dendritic processes. The experimental basis for this conclusion is, (1) dendritic impulses arising spontaneously or in response to electrical or mechanical stimulation arrive at the soma as depolarizing events of never more than 10–20 mV in amplitude (Figs 2–8); (2) impulses generated separately on the bilateral dendritic branches do not collide when converging simultaneously onto the apical neurite, but rather, they display temporal summation which may in turn trigger a propagated action potential in the cell’s axon (Figs 5–8); (3) whereas focal application of TTX to distal dendritic and axonal membrane rapidly blocks impulse generation in these regions, the toxin’s presence along the apical neurite has no effect on the ability of the dendrites or axon to generate action potentials. Significantly moreover, it has no effect on the passage of these potentials between the different topographical regions (Fig. 7). This indicates that dendro-axonal communication through the neurite is already operating via electrotonic conduction (see below).

In a neurone with AGR’s geometry, it is perhaps not surprising to find a functional separation of dendritic excitability into two discrete zones, one associated with each of the neurone’s bilateral receptor fields (see Fig. 10). As a consequence, mechanosensory information can be gathered and processed independently on the two sides, then transmitted across the apical neurite to the initial axon segment in a manner that is additive (‘AND’ gate) rather than mutually exclusive (‘OR’ gate). A uniform distribution of excitable membrane throughout the entire dendrite tree would not permit such regional integration to occur.

AGR’s dendrites still have the capacity to communicate directly with each other in that an impulse produced on one side may trigger an action potential in the dendritic branch of the opposite side. As suggested by Larimer & Kennedy (1966) for an homologous neurone in crayfish, a primary role of AGR could be to serve as a mixing circuit that balances accidental asymmetries in the action of the bilateral muscles with which the receptor is associated. Whichever terminal has the higher intrinsic excitability or is under the greater mechanical stress will dominate dendritic
output, its impulses crossing over to the opposite terminal and resetting the latter's pacemaker cycle.

Evidently this cellular mixing circuit is not hard-wired since during the course of an experiment, impulses arising on a given dendrite of AGR may drive its subordinate partner, while at other times, contralateral invasion and even the ability to activate the initial axon segment can fail spontaneously to occur. This variability in dendro-dendritic and dendro-axonal communication is potentially of major importance to the integrative capability of the cell. It may be that a facility to alter dynamically the input/output relationship of information transfer in this primary sensory neurone is conferred by modulatory influences such as those exerted upon the dendrites of central neurones (Llinás & Sugimori, 1980), their peripheral projections (Meyrand, Weimann & Marder, 1992) or even some form of auto-regulatory process (Llinás, Greenfield & Jahnsen, 1984).

**Dendritic morphology and excitability**

In principle, the lack of active impulse propagation on the apical neurite of AGR could be due to two general mechanisms. One possibility is that the ionophore composition (density and distribution of \( \text{Na}^+ \) channels) is such that the membrane is incapable of supporting a full action potential, as has been suggested for certain neurones of the vertebrate CNS (Huguenard et al. 1989). The other possibility is that the structural geometry of the apical neurite creates a region of low safety factor through which spikes may not propagate.

Blockade of impulse conduction in certain regions of neurones has been studied in a number of preparations (Wong & Pearson, 1975; Heitler & Goodman, 1978; Grossman, Parnas & Spira, 1979a; Smith, 1980; Gu, Muller & Young, 1991; Gu, 1991). In most cases, spike failure is associated with geometrical peculiarities that are also found in AGR, such as neuronal branching or a marked change in fibre diameter. These specialized regions are assumed to act as low pass filters to repetitive firing, allowing the passage of impulses at lower frequencies, but causing conduction block at higher rates of firing. From the physiological experiments reported here, however, it is clear that a different mechanism subserves the lack of active spike propagation in the apical neurite of AGR since spike failure occurs regardless of the frequency of incoming spikes, and not only at the point of bifurcation, but along the entire neuritic process, a distance of some 200–500 \( \mu \text{m} \). While our data do not exclude the possibility of an activity-independent contribution of branch point geometry to this conduction block, our experiments with focal TTX application strongly suggest that a paucity of functional \( \text{Na}^+ \) channels, at least in the neuritic membrane, is involved.

Do structural properties contribute to this compartmental inexcitability? In contrast to regions of AGR (dendrites and axon) that support action potentials, the apical neurite is not only considerably wider but has a complex membrane morphology, displaying membranal infoldings and planospiral-like coilings. One outcome of this unusual layering of AGR's neuritic membrane could be to limit the inward diffusion of sodium ions from the extracellular space, thereby reducing the probability of channel activation and spike generation. A corollary mechanism involving potassium accumulation in the periaxonal space is thought to lead to propagation failure in axons of other invertebrates (Grossman et al. 1979b), although
here again, impulse failure is frequency dependent rather than ‘pre-programmed’ as in the case of AGR.

A further consideration is the effect that the architecture of AGR’s apical neurite will have on passive cable properties of the compartment. While the relatively large diameter of this region presumably results in an elevated space constant for longitudinal current flow, the influence of membrane layering on electrotonic conduction is difficult to predict and awaits direct intracellular investigation.

Whether the purpose of the structural peculiarity of AGR’s neurite is to help create a region of non-excitable membrane (by preventing existing channels to function), or to ensure cable properties for effective electrotonic transmission across a membrane already lacking in Na⁺ channels therefore remains to be seen. Future experiments will also assess whether similar morphological specializations extend to other neurones of the stomatogastric nervous system that are known to possess passive membrane compartments (Nagy, Dickinson & Moulins, 1981).

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