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ELECTRICAL PROPERTIES OF INSECT NEURONES WITH SPIKING AND NON-SPIKING SOMATA: NORMAL, AXOTOMIZED, AND COLCHICINE-TREATED NEURONES

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SUMMARY

1. The paired motorneurones in the metathoracic ganglion of the locust have non-spiking somata whereas the dorsal unpaired media (DUM) neurones have spiking somata. We have studied the electrical properties of two identified neurones in the metathoracic ganglion that have spiking axons innervating the same muscle: the fast extensor tibiąe (FETi) motorneurone has a non-spiking soma and the dorsal unpaired median extensor tibiae (DUMETi) neurone has a spiking soma.

2. The inward current of the peripheral axon spikes of both DUMETi and FETi is carried predominantly by Na^+, since the spikes are blocked only by removal of Na^+ or addition of tetrodotoxin (TTX).

3. The inward current of the soma spike of DUMETi is carried by Na^+ and Ca^{2+}, since it is blocked by either removal of Na^+, addition of TTX, or addition of Co^{2+}.

4. The non-spiking soma of FETi shows delayed rectification. When some of the outward K^+ current is blocked by TEA or 3-AP, the soma is capable of generating overshooting action potentials. The inward current of the TEA-induced soma spike of FETi is carried by Na^+ and Ca^{2+}, since it is blocked by either removal of Na^+, addition of TTX or addition of Co^{2+}.

5. Axotomy or treatment with colchicine converts the non-spiking soma of FETi into a spiking soma within 4 days. The inward current of the soma spike is carried predominantly by Na^+, since it is blocked only by removal of Na^+.

6. Five days after axotomy of one of the peripheral axons of DUMETi, Na^+ is sufficient for the generation of the soma spike. Increased excitability is also observed in the neuropil of DUMETi after axotomy.

7. When some of the outward K^+ current is blocked by Ba^{2+}, the normal and axotomized somata of FETi and DUMETi are all capable of generating long duration Ba^{2+} action potentials that are blocked by addition of Co^{2+}. The overshooting Ba^{2+} action potentials in all cases are similar in amplitude and duration.

8. It is concluded that the soma membrane of DUMETi and FETi normally contains both Na^+ and Ca^{2+} inward current channels. The normal difference in excitability between these two somata may result in part or entirely from differences in the outward K^+ current. It is suggested that

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axotomy or colchicine treatment cause an increase in the number of active Na^+ channels in the soma membrane, which overcomes any differences in the outward K^+ current and results in both cells being able to produce soma spikes.

INTRODUCTION

Different neurones and separate regions of the same neurone can have either electrically excitable or electrically passive membrane with a quantitative and qualitative variety of inward and outward current channels. Three general types of cells have been described among the identified neurones of the insect central nervous system: (i) 'non-spiking' neurones with non-spiking neuropil processes and without a spiking axon, (ii) 'spiking' neurones with spiking axons and non-spiking somata, and (iii) 'spiking' neurones with spiking axons and spiking somata. Extensive recordings from identified neurones with axons leaving the ganglion (motorneurones and interganglionic interneurones) show that the majority of such neurones have spiking axons and non-spiking somata (Hoyle & Burrows, 1973; Burrows, 1977). Attenuated axon spikes only passively invade these somata from the neuropil. The largest (mean diameter 80 μm) and most accessible of these somata is that of the fast extensor tibiae (FETi) motorneurone, which innervates the large muscle providing the power for the locust jump (Hoyle & Burrows, 1973; Fig. 1 A). However, a special group of neurones along the midline on the dorsal surface of the thoracic ganglion have somata which are capable of generating overshooting action potentials. Because each of these neurones is unpaired and has bilaterally symmetrical axons, they are called dorsal unpaired median (DUM) neurones (Hoyle *et al.* 1974). One of the largest of these somata (50 μm diameter) in the metathoracic ganglion sends axons out both sides of the ganglion to the extensor tibiae (ETi) muscle (Hoyle *et al.* 1974), the same muscle innervated by FETi. It is the only DUM neurone to do so, and hence is an identified neurone (DUMETi; Fig. 1 A).

We are interested in three questions concerning the differences in excitable and passive membranes of identified neurones of insects. (i) What are the differences in ionic current channels and electrical properties between different neurones and separate regions of the same neurone? (ii) Are these differences plastic; are there experimental techniques whereby we can change the electrical properties of neural membrane? (iii) Can these differences be related to differences in function of the neurones? We have approached these questions by studying the electrical properties of FETi and DUMETi in normal adult animals and in animals in which the peripheral axons of both neurones were cut or treated with colchicine.

METHODS

Preparation. Adult male and female locusts (*Schistocerca nitens*) were obtained from crowded laboratory colonies maintained at 32 °C and 50% humidity, with 16/8 h light/dark cycle. The fast extensor tibiae (FETi) motorneurone and the dorsal unpaired median extensor tibiae (DUMETi) neurone were used in this study. A preparation similar to that described by Hoyle & Burrows (1973) was used to expose the ventral surface of the ganglion and record from the soma of FETi. A preparation

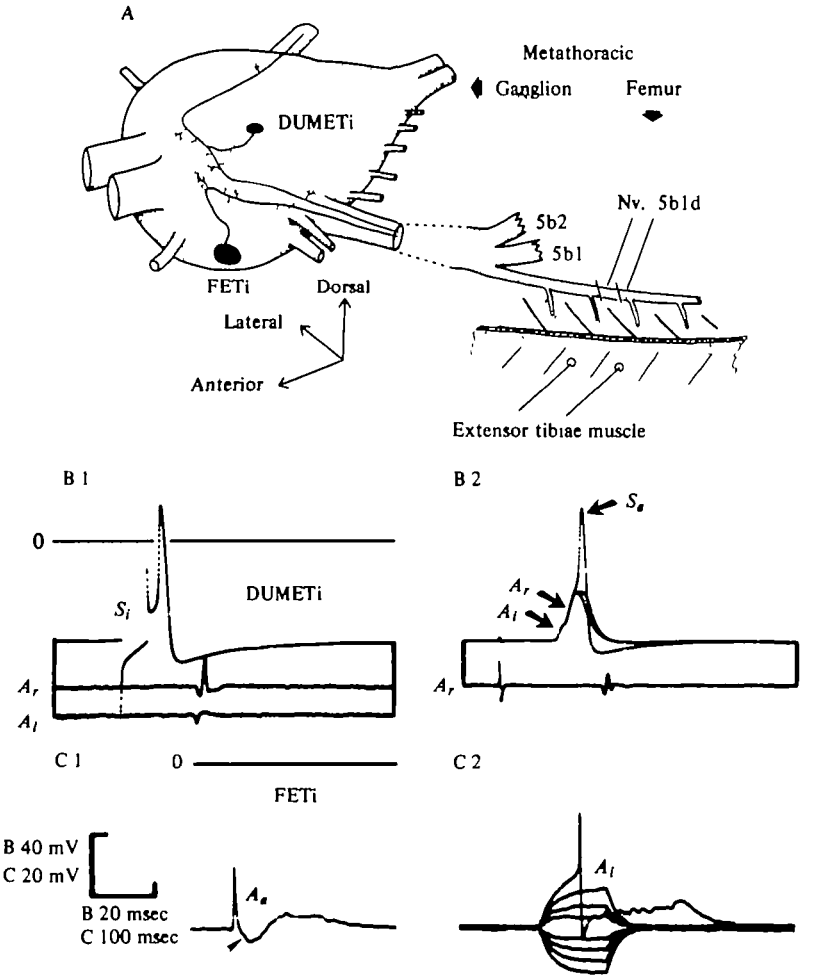

Fig. 1. (A) The anatomy of the dorsal unpaired median extensor tibiae (DUMETi) neurone and the fast extensor tibiae (FETi) motoneurone as revealed by cobalt sulphide staining, drawn semi-diagrammatically from whole-mount preparations of the metathoracic ganglion. FETi is a paired neurone (only the left one shown) with a soma (80 μm diameter) on the ventral lateral surface; DUMETi is an unpaired neurone with a soma (50 μm diameter) on the dorsal median surface. Recordings were made, and stimuli applied, through glass microelectrodes in the somata, steel hooks on the peripheral nerves, and copper wires in the muscle.

(B) DUMETi has a spiking soma and spiking axons. Modes of stimulation and spike components in DUMETi. (B 1) A short duration low amplitude pulse of current (5 ms, 2 nA) injected into the soma initiated an overshooting soma (S_i) spike (second trace). A zero reference potential is shown (upper trace). Axon spikes were recorded extracellularly in the right (third trace) and left (lower trace) peripheral axons. (B 2) The left peripheral axon was stimulated (note artifact) and recordings made from the right peripheral axon (lower) and the soma (upper). Three stimuli were superimposed. The first initiated a soma (S_a) spike and a right peripheral axon spike, the second and third initiated only a double component axon spike (A_l and A_r) and failed to initiate a soma spike. (C) FETi has a spiking axon and a non-spiking soma. (C 1) Axon spike (A_a) evoked by antidromic stimulation of the peripheral axon and recorded in soma. Arrow marks end of afterpotential and onset of reflex evoked IPSP followed by EPSPs. (C 2) Axon spike (A_i) evoked by injection of current into soma. Voltage is measured with a second microelectrode in the soma, current pulses are $\pm$1–4 nA for 100 ms. Note reflex evoked EPSPs after spike. A zero reference potential is shown.

Table 1. *Composition of salines (mM except where noted), pH 7·2*

Saline	NaCl	KCl	$CaCl_2$	Choline-Cl	Tris-HCl	$CoCl_2$	$BaCl_2$	TEA-Br	3-AP	$LaCl_3$	TTX (M)	TES
Normal	140	10	5	—	—	—	—	—	—	—	—	5
Na^+-free, choline	—	10	5	140	—	—	—	—	—	—	—	5
Na^+-free, Tris	—	10	5	—	140	—	—	—	—	—	—	—
Co^{2+}	140	10	—	—	—	10–30	—	—	—	—	—	5
50 % Na^+	70	10	5	70	—	—	—	—	—	—	—	5
50 % Na^+, Co^{2+}	70	10	5	70	—	30	—	—	—	—	—	5
Ba^{2+}	140	10	5	—	—	—	10	—	—	—	—	5
Ba^{2+}, Na^+-free	—	10	—	90	—	—	50	—	—	—	—	5
Ba^{2+}, Na^+-free, Co^{2+}	—	10	—	90	—	10–30	50	—	—	—	—	5
TEA	90	10	5	—	—	—	—	50	—	—	—	5
TEA, Na^+-free choline	—	10	5	90	—	—	—	50	—	—	—	5
TEA, Na^+-free, Tris	—	10	5	—	90	—	—	50	—	—	—	—
TEA, Co^{2+}	90	10	—	—	—	10–30	—	50	—	—	—	5
3-AP	140	10	5	—	—	—	—	—	2	—	—	5
La^{3+}	140	10	5	—	—	—	—	—	—	2	—	5
TEA, La^{3+}	90	10	5	—	—	—	—	50	—	2	—	5
TTX	140	10	5	—	—	—	—	—	—	—	10^{-6}	5
TEA, TTX	90	10	5	—	—	—	—	50	—	—	10^{-6}	5

similar to that described by Heitler & Goodman (1978) was used to expose the dorsal surface of the ganglion and record from the soma of DUMETi. In both cases the metathoracic ganglion was left in the thoracic cavity and connected via nerves 5 (nomenclature of Pringle, 1939) to the metathoracic legs for purposes of identifying the somata of the two neurones, but otherwise isolated from the rest of the nervous system. The penetrations of a given soma often remained stable for several hours, an important characteristic given that each animal contains only one DUMETi neurone. We desheathed the preparation only in those experiments involving perfusion with tetrodotoxin (TTX). The thoracic cavity had a volume of less than 1 ml and was continuously perfused with saline (see *solutions* below) at a rate of 3–6 ml/min. All experiments, unless otherwise stated, were performed at room temperature (22 °C).

Intracellular recording. Intracellular recordings were made from FETi and DUMETi by impaling the somata with either one or two glass microelectrodes filled with 3 M potassium acetate having resistances of 30–50 MΩ. In those experiments using single electrode penetrations, current could be injected into the neurones through the electrode via a bridge circuit. In those experiments on FETi using double electrode penetrations, current was injected into the neurone through one electrode and voltage was measured with the second electrode. Current was monitored with a virtual ground circuit. The duration of action potentials recorded intracellularly in spiking membrane was measured as the time between maximum rate of rise and the maximum rate of fall. In non-spiking membrane duration was measured as the time between maximum rate of rise and the point of 50% decay in amplitude. In the figures, membrane potential is only referenced to absolute potential where explicitly stated.

Identification, extracellular stimulation and recording. A pair of 50 μm diameter copper wires, insulated up to the tips, was implanted in the extensor tibiae muscle (ETi). The soma of the FETi motorneurone was identified by recording the 1:1 antidromic action potentials evoked by stimulating the ETi muscle to twitch. The soma of FETi is the largest in the ganglion and can often be seen through the ventral sheath in a characteristic lateral position. The soma of DUMETi can not be seen through the dorsal sheath. The position of the soma of DUMETi was extremely variable, and finding it involved making more-or-less random penetrations over about two thirds of the dorsal surface of the ganglion. Once penetrated, DUMETi could be distinguished from other DUM cells by correlating spikes recorded intracellularly with spikes recorded extracellularly from the peripheral axons of DUMETi, and by recording the 1:1 antidromic action potentials evoked by stimulating one of its peripheral axons. These axons occur in nerve 5b1d (by analogy with the mesothoracic nervous system described by Campbell, 1961) which runs across the ventral surface of the ETi muscle. Nerves 5b1d on both sides were recorded and stimulated using paired stainless steel hook electrodes, with one pair of hooks (usually on the left nerve) often used to stimulate the nerve antidromically and the other pair of hooks (usually on the right nerve) used to record the orthodromic spike coming out of the extensor nerve on the other side of the animal. Nerve 5b1d contains the axons of only four neurones: DUMETi, FETi, the slow extensor tibiae (SETi), and the common inhibitor (CI) motoneurone. DUMETi is the only neurone of these four to have its soma on the dorsal surface of the ganglion, and it is also the only neurone of these four that is unpaired and thus has an axon in both nerves 5b1d.

Nerve section and colchicine application. One metathoracic leg was induced to autotomize in a newly molted (teneral) adult by pinching the leg, thus severing nerve 5 which contains the axons of both FETi and DUMETi. The somata were penetrated 4–5 days after axotomy. FETi was identified by its characteristic position, physiological properties, and antidromic spike evoked by stimulation of the proximal stump of nerve 5 within the thoracic cavity. DUMETi was identified by antidromic stimulation of nerve 5b1d on the intact side (Fig. 1). A concentration of 1% colchicine (Sigma) was dissolved in a heated solution of 2% agar in distilled water and cooled. Pieces of agar and colchicine mixtures were cut to approximately 0·5 × 1·0 mm. The pieces were inserted through a slit made in the exoskeleton of the femur between the flexor and extensor muscle so that they lay next to the extensor nerve. The incision was sealed with low melting point wax. The soma of FETi was penetrated 4–5 days after colchicine application. The soma of FETi was identified by antidromic spikes evoked by stimulating the ETi muscle, although the muscle itself often did not twitch after colchicine treatment.

Solutions. The normal and experimental salines are listed in Table 1. Most solutions were buffered to pH 7·2 using 5 mM-TES buffer (Sigma) with the addition of several drops of NaOH (or KOH in the Na^+-free salines). Sodium was replaced by choline chloride or Tris HCl (without TES buffer) in Na^+-free salines. Three pharmacological agents used were tetraethylammonium bromide (Eastman Organic), tetrodotoxin (Calbiochem), and 3-aminopyridine (Sigma); 3-AP solutions were mixed fresh in the morning of each experiment. Because the neural sheath was left intact there was considerable variability in the diffusion time required for different ions and pharmacological agents. The somata, located directly under the neural sheath, usually were affected before the neuropil processes. The speed of diffusion was greatly facilitated by cutting the connectives and nerves close to the ganglion. The thickness of the neural sheath, a factor partly dependent upon the age of the animal, appeared to be important; younger adults had thinner sheaths and required shorter perfusion time for ions to take effect. Different ions penetrate the ganglion with different speeds. For example, barium ions often took effect in less than 5 min, cobalt ions took effect in 5–10 min, while TEA often required 15–20 min to take effect. The intact ganglion seemed able to regulate calcium ions, at least for the duration of our experiments, and thus we were unable to examine the effect of various calcium ion concentrations on spike amplitude.

Action potential abbreviations. In a previous paper (Heitler & Goodman, 1978), three types of action potentials were distinguished in DUMETi: a soma spike (S) of 60–90 mV, a neurite spike (N) of 20–40 mV, and an axon spike (A) of 8–15 mV. (The neurite was defined as the link segment between the branch point and the soma as shown in Fig. 1.) Furthermore, the A-spike was shown to be composed of two discrete components, the A-spike in the left axon (A_l) and the A-spike in the right axon (A_r).

In this paper, the S-spike and A-spike abbreviations will also be used in reference to the soma and axon of FETi. Four further distinctions are made based on the four different methods used to evoke action potentials in DUMETi and FETi. Those action potentials recorded in the soma resulting from extracellular antidromic stimulation of a peripheral axon are given a subscript a (as in S_a or A_a), those action potentials recorded in the soma are evoked by intracellular injection of a current pul into the soma are given a subscript i (as in S_i or A_i), those action potentials recorded

in the soma resulting from antidromic stimulation of a peripheral axon in addition to intracellular injection of constant subthreshold depolarizing or hyperpolarizing current into the soma are given the designations S_a+I or A_a+I, and those action potentials recorded in the soma resulting from synaptic input in the neuropil are given a subscript s (as in S_s or A_s). Examples are shown in Fig. 1 B.

RESULTS

Normal DUMETi

Soma and axon spikes of DUMETi. A soma spike (S_i) could be elicited in DUMETi by injection of small depolarizing currents (1–3 nA) across the somatic membrane (Fig. 1 B). This stimulation regime, under most circumstances, caused axon spikes in both branches of the neurone, and these could be recorded extracellularly in the periphery. A soma spike (S_a) could also be initiated by electrical stimulation of the peripheral axon on one side. This caused an antidromic spike to propagate centripetally to the branch point within the ganglion, then centrifugally down the contralateral branch and simultaneously somatopetally up the neurite to the soma (Fig. 1 B). A spike which successfully propogated across the branch point could be recorded extracellularly from the contralateral peripheral axon. Stimulation at frequencies greater than 5 Hz often caused soma spike failure, revealing a waveform (A_a) which fractionated into two discrete components corresponding to spikes in the two peripheral axon branches (Fig. 1 B). With continued stimulation, the antidromic spike often failed to propagate across the branch point to the contralateral axon, and then only a single component waveform (A_l or A_r) was recorded in the soma.

Resting potentials from stable recordings of DUMETi varied from -40 to -60 mV. The input resistance (R_{in}) of the soma of DUMETi was measured by injecting small pulses of current ($< 10^{-9}$ A) through the recording electrode with the bridge in balance. Input resistances were found to range from 16 to 20 MΩ (mean 18, four cells). The soma spike of DUMETi had a duration of 3–7 ms (mean 4·3, twelve cells), an amplitude of 60–90 mV (mean 74·4), and an afterpotential of 7–20 mV (mean 14·8). We may contrast this with the axon spike, for example, of the descending contralateral movement detector (DCMD) interneurone (Rowell, 1971) which has a similar amplitude and hyperpolarizing afterpotential, but a duration of only 0·6 ms. We did not attempt to record intracellularly from the axons of DUMETi owing to their small size (< 5 μm). Thus, we do not know the real duration of the axon spike of DUMETi; axon spikes recorded in the soma are attentuated in amplitude and probably enhanced in duration due to electrotonic propagation. The single component axon spike of DUMETi had a duration of 5–10 ms (mean 7·3, 6 cells), an amplitude of 8–15 mV (mean 7·2), and an afterpotential of 0–6 mV (mean 2·2).

Ionic dependence of the axon spike of DUMETi. The ionic basis of the currents producing the axon and soma spikes was determined by changing the concentrations of extracellular ions and by adding various blocking agents to the normal saline. The following evidence suggests that most of the inward current of the axon spike of DUMETi is carried by Na^+. First, replacement of Na^+ with choline eliminated the axon spike (Fig. 2 A), even when increased stimulating currents were applied either intracellularly into the soma or extracellularly on the peripheral axon. During the time

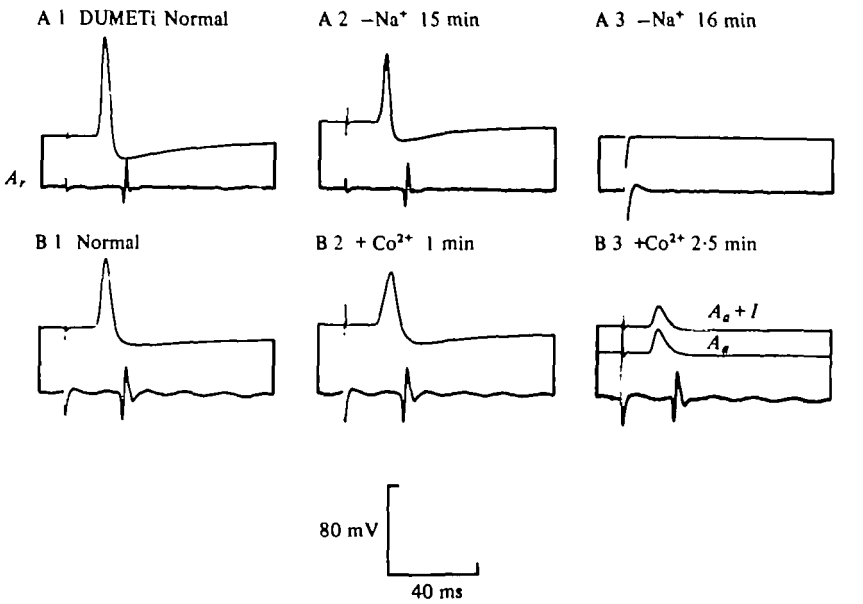

Fig. 2. Ionic dependence of soma spike and axon spike of DUMETi. The left peripheral axon was stimulated, and recordings made from the right peripheral axon (lower trace) and soma (upper trace). (A 1–3) The soma spike and axon spike were abolished by removal of Na^+ (a current pulse of 6 nA for 100 ms did not elicit a soma spike: cf. Fig. 1 B). The apparent depolarization in A 2 is caused by a change in the position of the oscilloscope trace. (B 1) Soma spike returned after addition of normal saline with Na^+. (B 1–3) The soma spike but not the axon spike was abolished by addition of Co^{2+}. (B 3) The soma spike was not elicited in Co^{2+} by antidromic stimulation of the axon spike (middle trace), antidromic stimulation of the axon spike superimposed on a 2·5 nA depolarizing current injected into the soma (upper), or by injection of over 5 nA current for 100 ms into the soma (1 nA evoked an axon spike).

period after the start of perfusion but before the axon spike was completely abolished transition spikes of reduced amplitude were recorded (see, e.g. Heitler & Goodman, 1978; Fig. 9), indicating that the effect of zero sodium is not merely to cause an increase in spike threshold. Second, addition of 10^{-8} M tetrodotoxin blocked the axon spike. Third, addition of 30 mM-Co^{2+} (Fig. 2 B) or 2 mM-La^{3+} for more than 20 min did not abolish the axon spike and had little effect on either the shape of the extracellularly recorded axon spike or the amplitude and duration of the intracellularly recorded axon spike as electrotonically propagated into the soma from the axon. Co^{2+} and La^{3+} have been demonstrated to block voltage-dependent Ca^{2+} current in other systems (Hagiwara & Naka, 1964; Hagiwara & Nakajima, 1966; Hagiwara & Takahashi, 1967; Hagiwara, Hayashi & Takahashi, 1969), and effected the soma spike of DUMETi in less than 5 min (see below).

Ionic dependence of the soma spike of DUMETi. The following observations indicate that, in normal saline, the inward current of the soma spike of DUMETi is carried by both Na^+ and Ca^{2+}. First, replacement of Na^+ with either choline or Tris eliminated the soma spike (Fig. 2 A). Transition soma spikes of reduced amplitude were recorded before the soma spike was totally abolished (Fig. 2 A). The effect of Na^+-free saline was reversed by washing the preparation with normal saline (Fig. 2 B). Second addition of 10^{-8} M tetrodotoxin abolished the soma spike, although the block to long

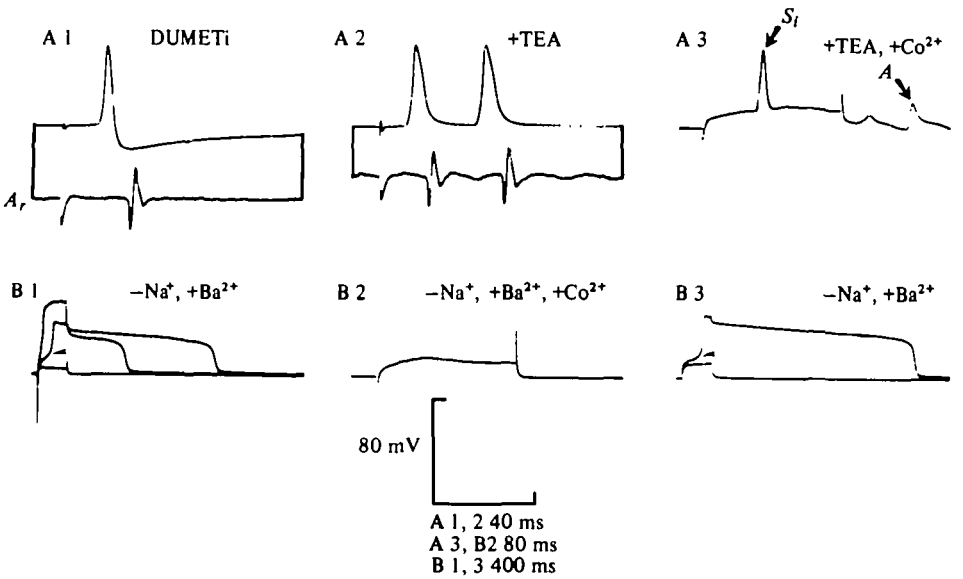

Fig. 3. (A) Effects of TEA on soma spike of DUMETi. (A 1) Normal soma spike. The left peripheral axon is stimulated and recordings made from the soma (upper trace) and right peripheral axon (lower trace). (A 2) TEA increased duration and abolished afterpotential of soma spike. Note repetitive firing of soma spike and repetitive firing of axon spike. (A 3) Injection of less than 1 nA into the soma evoked a soma spike in TEA plus Co^{2+}; Na^+ was sufficient for generation of a soma spike in TEA. (B) Ba^{2+} dependent action potentials in soma of DUMETi. (B 1) Ba^{2+} action potentials. Three superimposed sweeps in each 2 nA of current was injected into the soma for 50 ms (bridge circuit only approximately balanced). The first stimulus did not evoke a Ba^{2+} spike whereas the second and third did. There was a decrease in amplitude and duration with two successive suprathreshold (arrow) stimuli. (B 2) The Ba^{2+} action potential was reversibly blocked by the addition of Co^{2+}; injection of over 5 nA did not evoke an action potential. (B 3) Ba^{2+} action potential returned after Co^{2+} was washed out (two superimposed stimuli of 2 nA, one sub- and the other suprathreshold).

exposures was often not reversible over a 10 min wash. Third, addition of 30 mM-Co^{2+} (10 mM-Co^{2+} was often sufficient) abolished the soma spike (Fig. 2B). Addition of 2 mM-La^{3+} also abolished the soma spike. A current pulse of 1·4 nA was sufficient to elicit a soma spike after washing the preparation with normal saline for 8 min. Transition soma spikes of reduced amplitude and increased duration were recorded before complete abolition and during recovery (Fig. 2B).

Similar results were obtained in experiments on six DUMETi neurones. During the course of these experiments, we recorded from a variety of other DUM neurones, whose identities were unknown except for the knowledge that they were not DUMETi. The somata of these other DUM neurones also had active action potentials whose duration was more than 2 ms. Our observations indicate that, in normal saline, the inward current of the soma spike in other large DUM neurones also is carried by Na^+ and Ca^{2+}. In most of these DUM neurones, perfusion with either Na^+-free or Co^{2+} saline abolished the soma spike. In a few cases, however, the soma spike persisted in either the absence of Na^+ or the presence of Co^{2+}, but it was blocked by both treatments applied simultaneously. Thus, in a few of these other DUM neurones, one or both of the ions (Na^+ and Ca^{2+}) may alone have been sufficient to generate the inward current for an action potential, although the inward current of the soma spike in

normal saline is carried by both cations. Presumably this difference was due to either a different density of current channels, input resistance, or neuronal geometry.

Effects of TEA on soma spike of DUMETi. Normally both Na^+ and Ca^{2+} inward currents are necessary for the generation of a soma spike in DUMETi. Can inward current flowing through only the Na^+ channels be sufficient for the generation of an action potential in DUMETi if we block some of the outward K^+ current? To answer this question, the preparation was perfused with TEA plus Co^{2+} saline (Table 1). In other cells TEA appears to block a voltage dependent increase in outward K^+ current (Hille, 1967; Stanfield, 1970). The addition of TEA alone had three effects on the soma spike of DUMETi consistent with a blockage of outward K^+ current (Fig. 3A): (i) TEA prolonged the action potential by 50–100% by producing a hump on the falling phase of the spike; (ii) TEA eliminated the hyperpolarizing afterpotential and (iii) TEA caused repetitive firing of the soma spike. The repetitive firing of the soma spike was correlated with repetitive firing of the axon spike (Fig. 3A).

When the preparation was perfused with TEA plus Co^{2+}, the electrotonically propogated axon spike failed to generate a soma spike in DUMETi. However, injection of a current pulse into the soma did elicit a soma spike (Fig. 3A), albeit of somewhat reduced amplitude and duration (possibly due to the saline containing 90 mM-Na^+ rather than 140 mM-Na^+). Thus when divalent cation channels were blocked by Co^{2+}, inward current carried by Na^+ alone was sufficient to elicit a soma spike if some of the outward K^+ current was blocked by TEA.

Barium soma spike of DUMETi. The converse to the above question was next asked. Can inward current flowing through only the divalent cation channels be sufficient for the generation of an action potential in DUMETi if we block some of the outward K^+ current? To answer this question, the preparation was perfused with Na^+-free plus Ba^{2+} saline. In other cells Ba^{2+} is a more efficient carrier of inward current than Ca^{2+} through the divalent cation channels, and it is a blocker of some of the outward K^+ current (Fatt & Ginsborg, 1958; Hagiwara & Naka, 1964; Hagiwara, Fukuda & Eaton, 1974). Perfusion with Ba^{2+} should thus amplify the regenerative response due to inward current carried by divalent cations. Perfusion with 10 mM-Ba^{2+} had similar effects on the soma spike of DUMETi to TEA, suggesting that Ba^{+2} does indeed block outward K^+ current.

DUMETi produced action potentials in Na^+-free plus 50 mM-Ba^{2+} saline which were likely to be soma responses because of their low threshold and overshoot (Fig. 3B). The duration of the action potentials ranged from 300 to 800 ms and the amplitude ranged from 30 to 45 mV (resting potential was -40 mV). These responses were reversibly abolished by the addition of Co^{2+}, suggesting that the inward current was flowing through divalent cation channels (Fig. 3B). Repetitive stimulation of the cells at rates from 0·1 to 0·5 Hz caused a decrease in duration and a decrease in maximum amplitude of the Ba^{2+} action potential (Fig. 3B) consistent with a decline in the Ba^{2+} concentration gradient. In the absence of repeated stimuli for periods of 2 min or more, the duration and amplitude recovered to approximately their initial values. The final termination of the Ba^{2+} action potentials occurred at a faster rate than the plateau rate of decay, suggesting an active repolarization. This repolarization occurred at a constant potential about 20 mV positive of resting potential that was independent of the changes in duration or amplitude of the action potential during repetition.

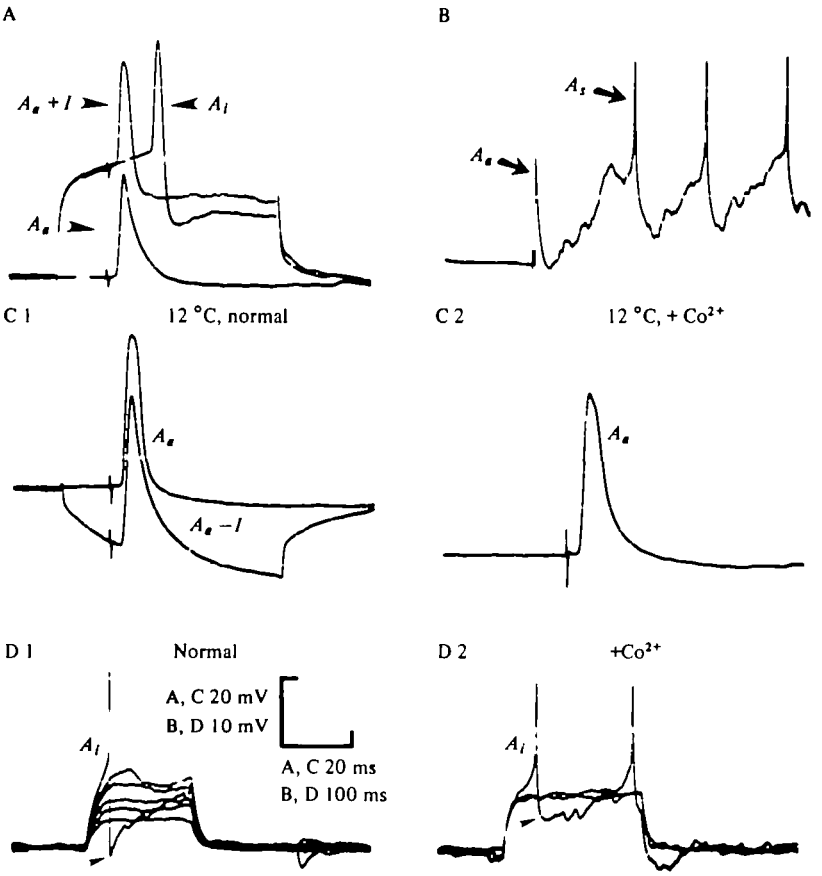

Fig. 4. Axon spikes recorded in the soma of FETi. (A) Three types of axon spikes recorded by a single microelectrode (bridge balanced) in the soma of FETi at room temperature (22 °C): axon spike (A_a) evoked by antidromic stimulation of peripheral axon; axon spike ($A_a + I$) evoked by antidromic stimulation superimposed on injection of depolarizing current (7 nA) into soma; and axon spike (A_I) evoked by injection of depolarizing current (7 nA) into soma. (B) Axon spike (A_a) evoked by antidromic stimulation followed by axon spikes (A_s) evoked by synaptic input from re-excitation reflex. (C) Same cell as A at 12 °C. (C 1) Axon spike (A_a) evoked by antidromic stimulation and axon spike ($A_a - I$) superimposed on injection of hyperpolarizing current (10 nA) into soma. (C 2) Antidromic axon spike (A_a) after perfusion with Co^{2+} saline. (D) Double electrode penetration of soma of FETi. (D 1) Injection of 2·0–4·5 nA (0·5 nA steps) for 150 ms. There is a slight indication of delayed rectification at 4·0 nA just below threshold for initiation of axon spike. Note large afterpotential (arrow). (D 2) Injection of 4·0, 4·5, and 5·0 nA for 200 ms. Same cell perfused with Co^{2+} saline. Note increased duration of spike and reduced afterpotential (arrow). All effects of Co^{2+} were reversible.

We conclude from these experiments that while under normal circumstances both Na^+ and Ca^{2+} currents are necessary to generate a regenerative response in the soma of DUMETi, either Na^+ or divalent cation (in our experiments Ba^{2+}) current alone can generate a regenerative response when some of the outward K^+ current is blocked.

Normal FETi

Axon spike of FETi. The somatic membrane of FETi does not normally support regenerative action potentials. Stimulation of the peripheral axon of FETi causes an action potential to propagate back towards the ganglion. The attenuated amplitude of this antidromic axon spike recorded in the soma of FETi could be as large as 30 mV; this is much greater than the antidromic spike recorded in most locust motorneurones (usually 2–5 mV). Penetrations of FETi often remained stable for several hours and had resting potentials of -40 to -60 mV as measured between the soma membrane and the extracellular space at the end of the experiment. The antidromic axon spike (A_a) of FETi, evoked by extracellular stimulation of the peripheral axon (Figs. 1C, 4A), had a duration of 2·5–5·0 ms (mean 3·3, seventeen cells), an amplitude of 11–31 mV (mean 21·1), and a hyperpolarizing afterpotential of 0–4 mV (mean 2·0).

A second stimulus regime used to evoke an axon spike in FETi was to inject a pulse of depolarizing current into the soma. This injected current elicited an axon spike (A_i) which was electrotonically propagated back into the soma (Figs. 1C, 4A). A third stimulus regime used to evoke an axon spike was synaptic excitation following an antidromic spike with the tibia restrained at 90° to the femur (Burrows & Horridge, 1974). These synaptically-driven axon spikes (A_s) were electronically propagated into the soma (Fig. 4B).

Ionic dependence of the axon spike. Most of the inward current of the axon spike of FETi is carried by Na^+. Perfusion with Na^+-free saline abolished the axon spike whereas perfusion with Co^{2+} did not.

Somatic delayed rectification of FETi. The axon spikes recorded in the soma differed markedly in duration and shape depending upon the stimulus regime used to evoke them. For example, in a typical preparation, the axon spike evoked by antidromic stimulation (A_a) had an amplitude of 19 mV, a duration of 3 ms, and an afterpotential of 2 mV (Fig. 1C). When an axon spike was elicited in the same cell by a current pulse injected into the soma, the amplitude was 18 mV, the duration 1·2 ms, and the afterpotential 20 mV (Fig. 1C). This phenomenon was first observed by Hoyle & Burrows (1973), who suggested that the somatic membrane was not completely passive, but rather that depolarization of the soma membrane either by the electrotonically propagated axon spike or by injected current caused an increase in outward K^+ current (delayed rectification). The results could be explained if the depolarization resulting from the axon spike (A_i) superimposed on the depolarization of the soma (required to evoke the axon spike) caused a greater increase in outward K^+ current than the depolarization resulting from the antidromic spike (A_a) alone (Figs. 1C, 4A). This is further supported by three other observations. First, axon spikes that were synaptically driven (such that the EPSPs caused a certain level of somatic depolarization upon which the electronic axon spike was superimposed) had a shorter duration and increased afterpotential than a preceding antidromic axon spike (Fig. 4B). Second, when antidromic axon spikes were superimposed on subthreshold depolarization, they had a shorter duration and larger afterpotential (Fig. 4A). Third, if the amplitude of the antidromic spike (A_a) was increased by cooling the preparation (Heitler, Goodman & Rowell, 1977) the duration markedly decreased below about 18 °C. At room temperature an antidromic spike had an amplitude of 30 mV and a

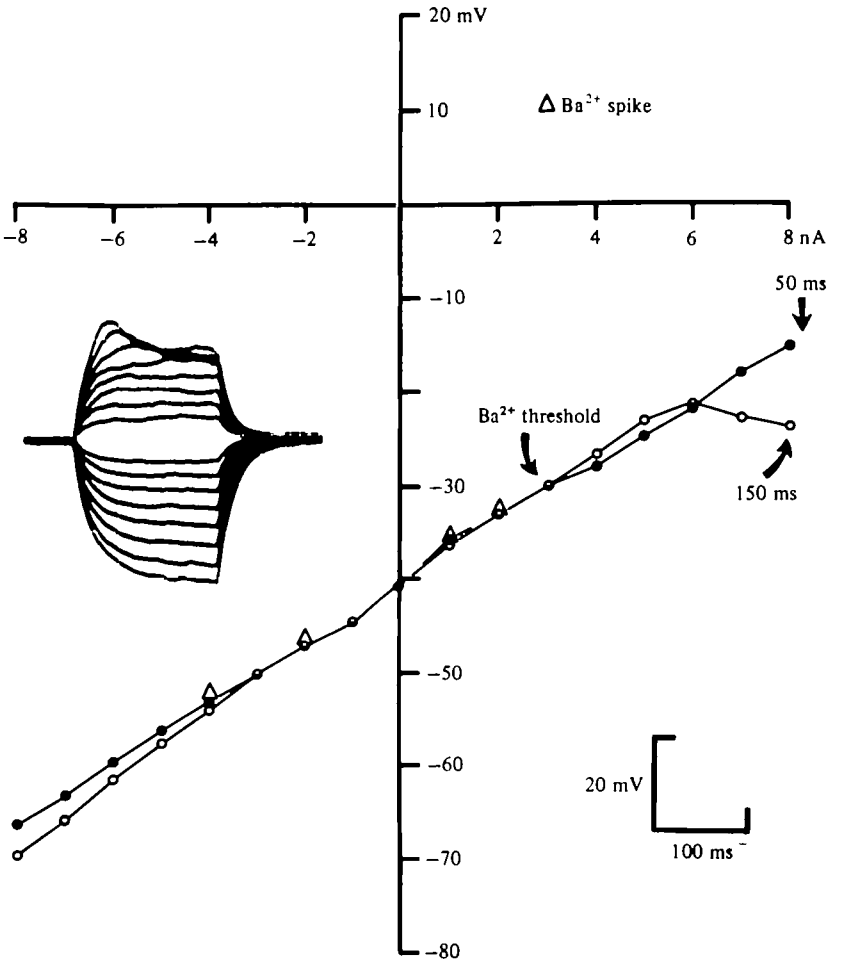

Fig. 5. Current/voltage relationship in soma of FETi from injection of constant current for 150 ms, recorded by double electrode penetration. In many cells, the *I*/V curve appeared linear at voltages before initiation of an axon spike. In this cell, however, resting potential was −40 mV and delayed rectification was observed before initiation of axon spike (at −22 mV). Filled circles show voltage after 50 ms; open circles show voltage after 150 ms. Insert shows delayed rectification in this cell by injection of 1–8 nA for 150 ms. When cell was perfused with Na^+-free plus Ba^{2+}, the resting resistance was unchanged (triangles). Threshold for Ba^{2+} action potential was −30 mV and the action potential was overshooting to +10 mV.

duration of 6 ms. At 12 °C the same antidromic spike had an amplitude of 45 mV and a duration of 4 ms. If this was then superimposed on a hyperpolarizing current pulse of 10 nA, the duration increased again to 6 ms (Fig. 4C).

We examined the delayed rectification of the soma FETi by penetration of the soma with two microelectrodes and measured the current/voltage (*I*/V) relationship by injection of constant current pulses. The threshold for spike initiation in the axon was often so low that the soma membrane appeared linear in its *I*/V relationship (Fig. 1C) (Heitler *et al.* 1977; Goodman & Heitler, 1977). When the threshold for axon spike initiation was high, however, evidence for delayed rectification was observed in the

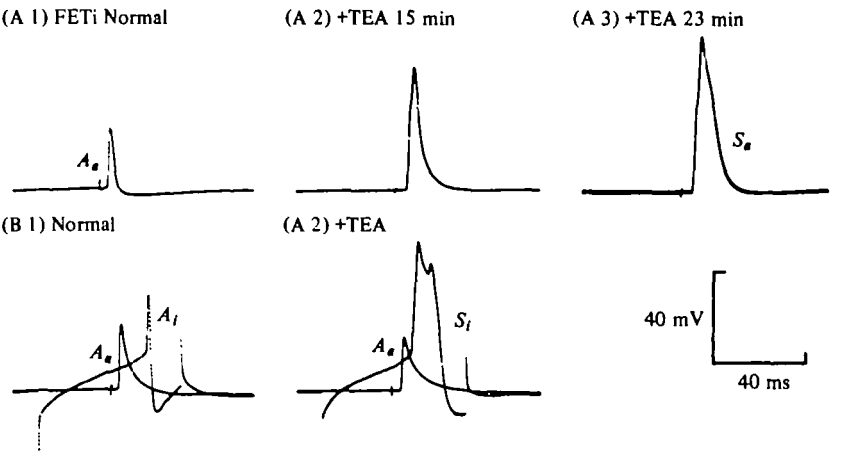

Fig. 6. Some spike in FETi induced by TEA. Soma was normally non-spiking. (A 1–3) Sequence in a single cell. (A 1) Antidromic stimulation evoked an axon spike in normal saline. (A 2–3) After addition of TEA, the antidromic axon spike evoked an overshooting soma spike. (B1–2) Sequence in a single cell. (B 1) Axon spike in normal saline evoked by antidromic stimulation (A_a) and by injection of 7 nA current into soma (A_i). (B 2) After perfusion with TEA, soma spike was elicited by injection of 10 nA current into soma (S_i). Antidromic axon spike (A_a) had increased duration but did not elicit soma spike.

soma (Fig. 5). The input resistance (R_{in}) of the soma of FETi had a mean of 3·3 MΩ (S.D. = 0·4, 8 cells). Delayed rectification at subthreshold depolarizations caused a reduction of R_{in} to about 2·0 MΩ.

The afterhyperpolarization following an axon spike produced by injected current could be reversibly reduced by over 50% by the addition of Co^{2+} (Fig. 4D). The duration of the large amplitude axon spikes (A_a) produced at low temperatures could be reversibly increased by the addition of Co^{2+} (Fig. 4C). These results suggest that some of the delayed rectification maybe a result of a Ca^{2+}-activated outward K^+ current (Meech & Standen, 1975).

Three lines of evidence support the notion that the delayed rectification was produced in the soma membrane rather than being the reflexion of delayed rectification in the neuropil process or peripheral axon. First, the magnitude of the response was large. An initial 25 mV depolarization of the soma resulted in a 10 mV hyperpolarization of the soma membrane. If this were not a response directly of the soma, then the attenuation of the initial depolarization out to the neuropil and the attenuation of the rectification back to the soma would suggest a rectification of enormous magnitude. Second, depolarization of the soma caused by synaptic input out in the neuropil had to be at least as great as the depolarization caused by current injected into the soma to cause a similar change in the duration and afterpotential of the axon spike as recorded in the soma. Considering the great attenuation of the neuropil depolarization in the soma and vice versa, we might expect that if the delayed rectification was a response in the neuropil, that much less synaptic depolarization would be required as recorded in the soma to cause the same effect on the axon spike. Third, the soma of FETi was capable of generating overshooting action potentials when perfused with agents that block outward K^+ current, as described below.

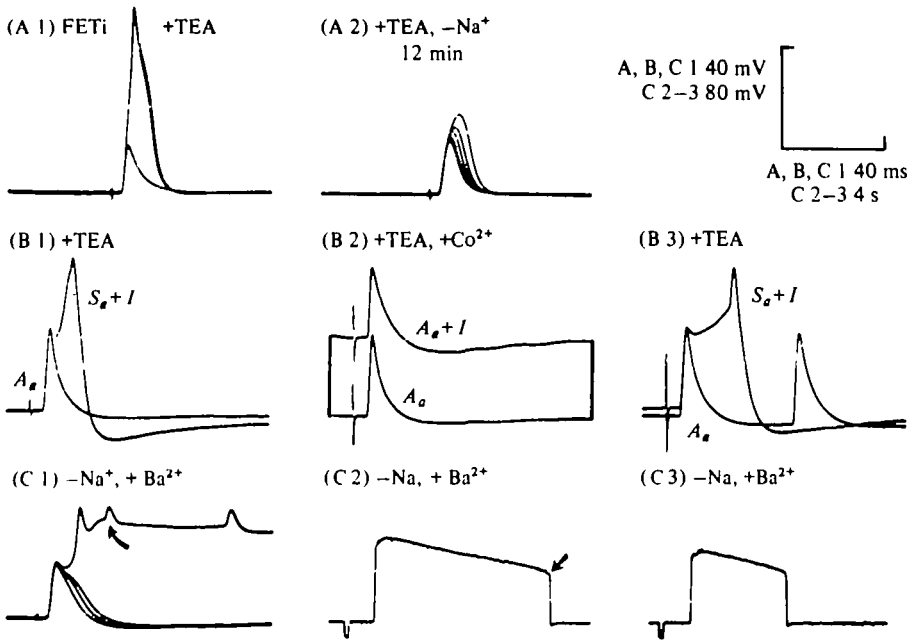

Fig. 7. (A, B) Ionic dependence of TEA-induced soma spike and axon spike of FETi. (A) TEA-induced soma spike and axon spike were abolished by removal of Na^+. (A 1) In TEA, antidromic stimulation evoked a soma spike in two of the three superimposed traces. (A 2) In TEA plus Na^+-free, the five superimposed traces show sequential reduction in amplitude of soma spike until only axon spike is left. Shortly after A 2, the axon spike too was reversibly abolished. Neither axon nor soma spike could be elicited with 30 nA depolarizing current injected for 100 ms. (B) TEA-induced soma spike was abolished by addition of Co^{2+} whereas axon spike was not. (B 1) TEA-induced soma spike evoked by antidromic stimulation superimposed on injection of 4 nA current into soma (second trace is antidromic axon spike without current). (B 2) TEA-induced soma spike was reversibly abolished in Co^{2+}; soma spike was not evoked by antidromic axon spike (lower trace) or antidromic axon spike superimposed on 7 nA current (upper). 30 nA depolarizing current injected for 100 ms also failed to elicit a soma spike. (B 3) TEA-induced soma spike was evoked by antidromic axon spike superimposed on 7 nA (upper trace) after Co^{2+} was washed out. Note repetitive firing of axon spike in lower trace (without current). (C) Ba^{2+} action potential in soma of FETi. (C 1) Four sequential superimposed traces of transitional soma responses to antidromic stimulation as the Ba^{2+} began taking effect before the Na^+ was washed out. Arrow marks Na^+ axon spikes superimposed on overshooting depolarization of soma. (C 2–3) Ba^{2+} soma action potentials in Na^+-free plus Ba^{2+}, evoked by 4 nA depolarizing current (150 ms) preceded by pulse of 4 nA hyperpolarizing current for 150 ms to measure R_{in}. Decrease in amplitude and duration in one cell for two successive stimuli at 0·5 Hz. Arrow marks repolarization potential.

Soma spike of FETi induced by TEA. Are inward current channels normally present but masked by outward current channels in the soma of FETi? Previous work in the cockroach (Pitman, 1975*b*) showed that a normally non-spiking soma was converted into a spiking soma by (i) perfusion with TEA, which blocked outward K^+ current, and (ii) injection of citrate ions or EGTA, which reduced the intracellular free calcium concentration (Pitman, 1975*b*), increasing the Ca^{2+} concentration gradient (and possibly also blocking any Ca^{2+}-activated outward current).

The soma of FETi produced overshooting action potentials after perfusion with TEA (Fig. 6). The diffusion of TEA across the ganglionic perineural sheath was slow and often required perfusion for 15–20 min. In a few cases, the antidromic axon spike

was sufficient to evoke a soma spike in TEA (Fig. 6A), but usually, the antidromic axon spike merely had an increased duration as recorded in the soma (Fig. 6B), due to either a partial somatic electrogenesis or to a prolonged axon spike in TEA, or both. In these cases, a soma spike could be elicited by direct intracellular injection of current pulses into the soma (Fig. 6B), or by superimposing the antidromic axon spike on a subthreshold depolarization of the soma. The soma spike of FETi produced in TEA had a duration of 8–20 ms (mean 10·2, ten cells, as measured between the maximum rate of rise and fall), an amplitude of 55–70 mV (mean 60·5), and a hyperpolarizing afterpotential of 1–10 mV (mean 5 mV). The presence of an afterpotential suggests that 50 mM-TEA did not block all of the outward K^+ current. The soma spike in TEA had a variety of shapes. In some cases, there was a hump on the falling phase of the spike (Fig. 6A) while in other cases, a second spike-like potential was recorded during the hump on the falling phase (Fig. 6B). This second spike-like potential could possibly be an axon spike electrotonically propagated back into the soma. The axon of FETi is capable of repetitive firing in TEA (Fig. 7B).

Perfusion with 2 mM 3-aminopyridine (3-AP) also evoked overshooting soma spikes in FETi. In other cells 3-AP appears to block a voltage-dependent increase in outward K^+ current (Pelhate & Pichon, 1974; Thompson, 1977). In FETi, perfusion with 3-AP also affected the resting potential by causing a slow depolarization which eventually led to spontaneous firing of the cell. Perfusion with TEA did not affect the resting potential for periods of over 30 min. Thus, 3-AP appeared also to block some of the outward K^+ current responsible for the resting potential whereas TEA did not.

Ionic dependence of the soma spike of FETi in TEA. The following evidence suggests that, in TEA solution, the inward current of the soma spike of FETi is carried by both Na^+ and Ca^{2+}. First, replacement of Na^+ with choline or Tris (Na^+-free plus TEA) eliminates the soma spike. Perfusion with Na^+-free plus TEA also abolished the axon spike. The effect of Na^+-free plus TEA was reversed by washing the preparation with TEA saline. Transition soma spikes of reduced amplitude were recorded, both as evoked by antidromic stimulation (Fig. 7A) and by current injection. Second, addition of 10^{-8} M-TTX abolished the soma spike in TEA. Third, addition of 30 mM-Co^{2+} (10 mM-Co^{2+} was often enough) or 2 mM-La^{3+} to the TEA solution abolished the soma spike in TEA within 10 min. Before the soma response was fully abolished, transitional responses were recorded in which the axon spike had a hump on the falling phase. The effect was fully reversed after washing the preparation with TEA saline, and the axon spikes, superimposed on 7 nA depolarizing current, evoked a soma spike (Fig. 7B).

These observations indicate that, in TEA saline, the inward current of the soma spike is carried by Na^+ and Ca^{2+}. This suggests that monovalent and divalent inward current channels are normally present in the soma membrane of FETi. The presence of these voltage-dependent inward current channels normally is masked by outward K^+ current. This is in contrast to the ionic dependence of the soma spike induced by TEA reported for the fast coxal depressor motorneurone of the cockroach (Pitman, 1975*b*). In this neurone, perfusion with TEA evoked a soma spike in which Ca^{2+} had the major role in carrying the inward current.

Barium soma spike of FETi. The soma of FETi produced action potentials in Na^+-free plus Ba^{2+} solution. During the first few minutes of perfusion, transitional responses were often observed in which the antidromic axon spike (dependent on Na^+)

elicited a long duration soma spike with a repetitive burst of up to 20 axon spikes superimposed on the long duration plateau. Perfusion for a few additional minutes with Na^+-free plus Ba^{2+} abolished the axon spikes. Injection of a current pulse into the soma now elicited an overshooting Ba^{2+} soma spike (Fig. 7C). The threshold and peak membrane potential of the Ba^{2+} spike from one experiment are shown in Fig. 5. The duration of the action potential ranged from 400 to 7500 ms, and the amplitude ranged from 35 to 70 mV (resting potential was -40 mV). These action potentials were reversibly blocked by the addition of Co^{2+}, suggesting that the inward current flowed through the divalent cation channels.

Repetitive stimulation of the cells at 0·05 Hz caused a decrease in duration and a decrease in maximum amplitude of the Ba^{2+} action potential (Fig. 7C). We measured the resting input resistance before each successive action potential by injecting a 4 nA hyperpolarizing current pulse for 150 ms and found that R_{in} remained unchanged at 2·9 MΩ. These results are consistent with a decline in the Ba^{2+} concentration gradient and suggest that the soma of FETi can not either sequester or pump large amounts of Ba^{2+} out of the intracellular compartment. The membrane potential at which repolarization occurred remained constant with repetitive stimulation. In the absence of repeated stimuli for periods of 5 min or more, the duration and amplitude of the action potential recovered to greater than 90% of its initial value, suggesting that in time the Ba^{2+} concentration gradient is restored.

Axotomized and colchicine-treated FETi

Non-spiking soma of FETi converted into a spiking soma. Cat spinal motorneurones react to axotomy by an increased electrical excitability in their dendrites (Kuno & Llinas, 1970). Axotomy or treatment with colchicine (Pitman, Tweedle & Cohen, 1972; Pitman, 1975*a*) converted the non-spiking soma of the fast coxal depressor motorneurones (D_f) in the cockroach within four days into a spiking soma capable of generating overshooting action potentials. It was concluded that Na^+ was the major carrier of inward current of the action potential induced by colchicine. Another study using locust rather than cockroach motorneurones, however, reported that the non-spiking soma of FETi was not converted into a spiking soma by axotomy (Horridge & Burrows, 1974). Rather, changes in the time constant of the soma of the axotomized axon were reported.

In view of these conflicting reports we have (i) re-examined the effects of axotomy on the soma of FETi in the locust by inducing autotomy of the methathoracic leg, and (ii) examined the effects of colchicine treatment on the soma of FETi by implanting pieces of agar with 1% colchicine along the peripheral nerve in the metathoracic leg. We examined the soma of FETi 5 days after both axotomy and colchicine treatment. The resting potential of the soma of FETi after axotomy was similar to normal (-40 to -60 mV). We were able to evoke an overshooting action potential in the soma of FETi five days after colchicine treatment (Fig. 8A, B) and five days after axotomy (Fig. 8E, F). Usually the antidromic axon spike alone was not sufficient to evoke a soma spike. In these cases, two stimulus regimes were used to evoke a soma spike: (i) injection of a suprathreshold depolarizing current pulse into the soma (Fig. 8A, F) or (ii) antidromic stimulation of the axon spike superimposed on injection of a subthreshold depolarizing current pulse into the soma (Fig. 8B, E).

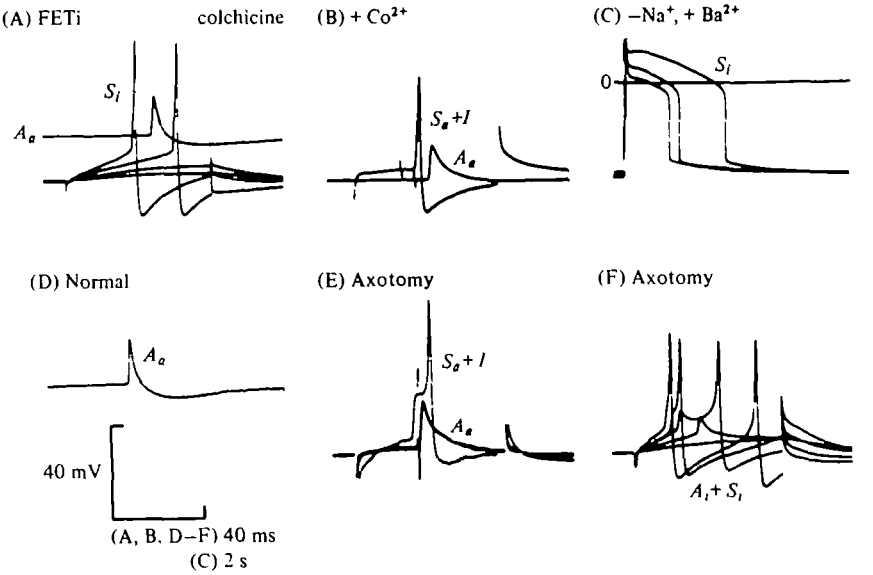

Fig. 8. Soma spike in FETi 5 days after colchicine treatment or axotomy. (A) Injection of current (1–4 nA) into soma evoked soma spike 5 days after colchicine treatment (lower traces), whereas antidromic stimulation of axon spike (upper trace) was insufficient. (B) Co^{2+} did not abolish colchicine-induced soma spike, in this case evoked by antidromic stimulation superimposed on 8 nA current injected into the soma (upper trace). (C) Three successive Ba^{2+} action potentials in soma of FETi 5 days after colchicine treatment (in Na^+-free plus Ba^{2+}). Note decrease in amplitude and duration upon repetition, but constant threshold of repolarization. Zero reference potential included. (D) Antidromic axon spike in normal FETi. (E) Increased duration of antidromic axon spike in FETi 5 days after axotomy. Soma spike was evoked by antidromic axon spike superimposed on 4 nA injected current. (F) Five days after axotomy, current injected into soma evokes axon spike before soma spike, as shown by five superimposed stimuli of 2, 4, 6, 8, and 10 nA for 60 ms.

The threshold for initiation of the axon spike to current injected into the soma was lower than for initiation of a soma spike, resulting in either a distinct axon spike preceding the soma spike (Fig. 8F) or an inflection on the rising phase of the soma spike (Fig. 8E, F). The soma spike in FETi induced by either axotomy or colchicine treatment had a duration of 1·0–2·0 ms (mean 1·3, seven cells), an amplitude of 40–90 mV (mean 59·1), and a hyperpolarizing afterpotential of 7–15 mV (mean 12·1). The R_{in} of axotomized FETi was 2·0 MΩ in two animals as opposed to a mean of 3·3 MΩ in the normal FETi.

Horridge & Burrows (1974) probably did not observe the soma spike because they only stimulated the soma by the antidromic axon spike and did not inject current into the soma. They reported an increased time constant by the observation of an increased duration in the falling phase of the antidromic axon spike. We often observed similar increases in the duration of the antidromic axon spike as a result of axotomy (compare Fig. 8D to Fig. 8E); this slower falling phase may be due in part to a subthreshold electrogenesis in the soma produced by the antidromic axon spike.

Ionic dependence of induced soma spike in FETi. The following observations indicate that, in normal saline, the inward current of the soma spike in FETi induced by either axotomy or colchicine treatment is carried predominantly by Na^+. First, replacement of Na^+ with choline eliminated the soma spike, even with injection of current pulses of

much greater amplitude and duration than are normally required to evoke a soma spike. The effect of Na^+-free saline was totally reversible. During the time period after the start of perfusion with Na^+-free saline and before the soma spike was totally abolished, transition soma spikes of reduced amplitude were recorded.

Second, perfusion with 30 mM-Co^{2+} (or 2 mM-La^{3+}) did not abolish the soma spike (Fig. 8B). In some cases, Co^{2+} caused a small decrease in amplitude and a small decrease in duration of the action potential (Fig. 8B). Most often, no effect was observed on either amplitude or duration. Perfusion with 50% Na^+ plus Co^{2+} reversibly abolished the soma spike. These results suggest the following: (i) Na^+ is a major carrier of inward current during the soma spike in FETi induced by either axotomy or colchicine, (ii) Ca^{2+} is not a major carrier of inward current during the soma spike in normal saline, and (iii) Ca^{2+} may carry part of the inward current during the soma spike, and this small Ca^{2+} inward current is necessary for the soma spike when the preparation is perfused with 50% Na^+.

Barium soma spike in FETi with induced soma spike. Since the soma spike of FETi produced by perfusion with TEA required both Na^+ and Ca^{2+}, whereas the soma spike in FETi induced by axotomy or colchicine treatment required only Na^+, we may ask whether the divalent cation inward current channels are still present and relatively unchanged in the soma of FETi after axotomy or colchicine treatment? To answer this question, the preparation was perfused with Na^+-free plus Ba^{2+} saline, under which conditions the soma of FETi 5 days after axotomy or colchicine treatment produced long duration action potentials (Fig. 8C). These Ba^{2+} action potentials were reversibly abolished by Co^{2+}, and had a threshold, amplitude, duration and overall waveform identical to Ba^{2+} spikes occuring in the soma of the normal FETi. These results suggest that the divalent cation inward current channels are still present in the soma of FETi after axotomy or colchicine treatment, and that they are relatively unchanged in their ability to produce long duration Ba^{2+} action potentials.

Other neurones, synaptic transmission, and axonal conduction after axotomy. The flexor tibiae motorneurones have peripheral axons in nerve 5 along with FETi. Five days after sectioning nerve 5, the somata of flexor tibiae motorneurones have been converted from non-spiking to spiking, in exactly the same manner as FETi. We have examined an excitatory pathway within the metathoracic ganglion from FETi to the flexor tibiae motorneurones (Hoyle & Burrows, 1973). Normally a spike in FETi causes a burst of spikes in the flexor tibiae motorneurones as a result of this central excitatory pathway (Heitler & Burrows, 1977). Five days after axotomy, an apparently similar burst of flexor spikes can be evoked by a soma spike in FETi. This suggests that there has been no dramatic change in either the central output of the FETi motorneurone or the input to the flexor tibiae motorneurones 5 days after axotomy of both FETi and the flexor tibiae motorneurones. Furthermore, the peripheral axons of FETi and the flexor tibiae motorneurones in the stump of nerve 5 are still capable of conducting action potentials 5 days after axotomy of the distal portion of nerve 5.

Axotomized DUMETi

Axotomy converts the non-spiking soma of FETi into a spiking soma. What effect does axotomy have on the already spiking soma of DUMETi? To answer this question, we axotomized one of the peripheral axons of DUMETi by autotomy of one of the

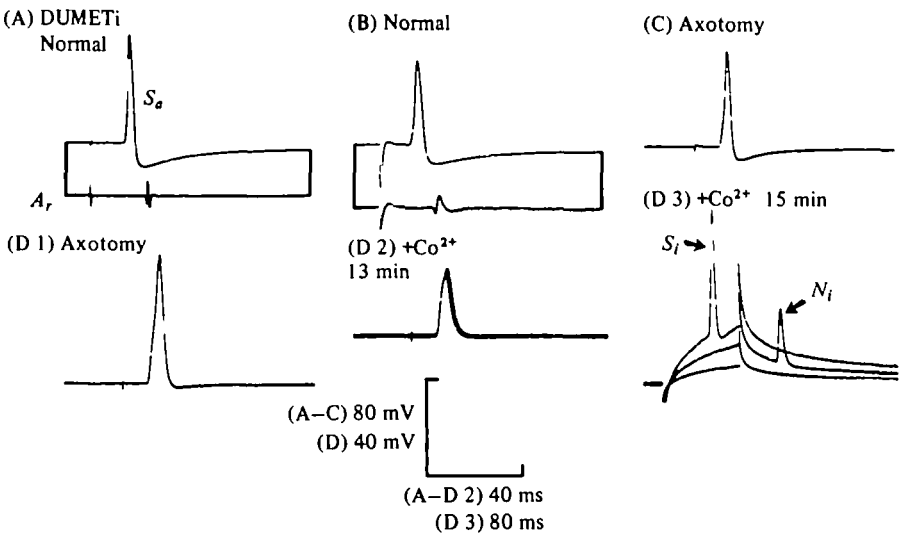

Fig. 9. Soma spike of DUMETi 5 days after axotomy of one of the peripheral axons. (A, B) Examples of normal soma spikes of DUMETi in two different preparations. (C) Soma spike in axotomized DUMETi was within the normal range in duration, amplitude, and afterpotential. (D 1–2) Co^{2+} abolished the ability of the antidromic stimulation to evoke a soma spike. (D 3) Injection of 1, 2, and 3 nA current pulses into the soma were subthreshold, evoked a neurite spike, and evoked a soma spike in Co^{2+}. The results suggest that Na^+ was sufficient to generate a soma spike in the axotomized DUMETi.

metathoracic legs and examined the soma spike 5 days later. The duration (3 ms), amplitude (74 mV), and afterpotential (12 mV) of the soma spike in the axotomized DUMETi were within the normal range (Fig. 9 A–C). In the normal DUMETi, Na^+ and Ca^{2+} inward currents were both necessary for the generation of a soma spike, neither ion alone was sufficient in normal saline. Perfusion of the axotomized DUMETi with Na^+-free saline reversibly abolished the soma spike when stimulated by both antidromic stimulation and injected current, just as in the normal DUMETi. However, perfusion of the axotomized DUMETi with Co^{2+} saline did *not* abolish the soma spike in response to injection of depolarizing current, unlike the normal DUMETi (Fig. 10D). One possible explanation is that Co^{2+} no longer blocks the Ca^{2+} channels after axotomy. However, addition of Co^{2+} did reversibly block the long duration Ba^{2+} action potential produced by perfusion with Na^+-free plus Ba^{2+} saline, just as in the normal DUMETi.

Thus, it appears that Na^+ is sufficient alone for the generation of a soma spike in DUMETi after axotomy. Furthermore, with subthreshold (for the soma spike) current injection in Co^{2+} (Fig. 9D), we only recorded a neurite spike. We were unable, even at high frequencies of repetitive stimulation, to fractionate the remaining action potential into component axon spikes. We interpret this to mean that the region of membrane about the branch point in which spikes may fail in the intact animal (see Heitler & Goodman, 1978) has now been converted into a fully active region of membrane in which spikes never fail, thus providing spike continuity between the two axon spike initiation sites and the neurite spike initiation site. It is tempting to suggest that th conversion of non-spiking membrane to spiking membrane in the neuropil of

DUMETi, the conversion of the soma spike of DUMETi from being sensitive to Co^{2+} to being resistant to Co^{2+}, and the conversion of the non-spiking soma of FETi to a spiking soma, all of which occur as a result of axotomy, are achieved by the same mechanism; namely, an increase in the number of active Na^+ channels.

DISCUSSION

We have examined the electrical properties of two identified neurones in the metathoracic ganglion of the locust: the fast extensor tibiae (FETi) motorneurone and the dorsal unpaired median extensor tibiae (DUMETi) neurone. Each of these two neurones has a peripheral axon capable of generating action potentials and in both cases the peripheral axons innervate the same muscle: the extensor tibiae (ETi) muscle of the metathoracic leg. FETi has a non-spiking soma and is representative of the bilaterally paired motorneurones and interganglionic interneurones with spiking axons and non-spiking somata, many of whose somata are located on the ventral surface of the ganglion. DUMETi has a spiking soma and is representative of the dorsal unpaired median (DUM) neurones with spiking axons and spiking somata. We have attempted to explain the differences in electrical properties of these neurones in terms of the relative distribution of ion channels in the different regions of their membrane.

The inward current of the peripheral axon spikes of both DUMETi and FETi is carried predominantly by Na^+, since they are blocked by removal of Na^+ or addition of TTX. There may be a Ca^{2+} component to these spikes, but it is not essential since they are not abolished by Co^{2+}. The inward current of the axon spikes in the ventral nerve cords of cockroaches and stick insects has similarly been shown to be carried predominantly by Na^+ (Yamasaki & Narahashi, 1959; Treherne & Maddrell, 1967). We did not record intracellularly from the peripheral axons of DUMETi or FETi, and thus do not know either the amplitude, duration, or afterpotential of the axon spikes. For comparison with soma spikes, however, we did record intracellularly from the axon of the descending contralateral movement detector interneurone in the metathoracic ganglion. A summary of our results is given in Table 2.

Electrical properties of DUMETi soma

The duration of the soma spike of DUMETi is several times longer than the duration of Na^+ axon spikes in most locust neurones (Table 2). The inward current of the soma spike is carried by Na^+ and Ca^{2+}, since it is blocked by removal of Na^+, addition of TTX, or addition of Co^{2+}. When some of the outward K^+ current is blocked by TEA, the mean duration increases and the hyperpolarizing afterpotential is totally abolished. Perfusion with TEA plus Co^{2+} produces a Na^+ soma spike, suggesting separate monovalent and divalent ionic channels. Perfusion with Na^+-free plus Ba^{2+} produces a long duration Ba^{2+} action potential which is reversibly blocked by Co^{2+}.

Jego *et al.* (1970) previously studied the ionic mechanism of action potentials from unidentified spiking somata on the dorsal surface of the sixth abdominal ganglion of the cockroach *Periplaneta americana*. By comparison with the work of Crossman *et al.* (1971), analogy with locust ganglia, and their own account, the somata penetrated by Jego *et al.* were likely to be those of the dorsal unpaired median (DUM) neurones.

Table. 2. *Mean amplitude, duration, afterpotential, and ionic basis of action potentials recorded in DUMETi, FETi, and DCMD in relationship to part of neurone, history of neurone, and saline*

Cell	Part	History	Saline	*n*	Action potential: Amplitude (mV)	Action potential: Duration (ms)	Action potential: Afterpotential (mV)	Predominant inward current
DCMD	Axon	Normal	Normal	2	85	0·6	12	Na^+
DUMETi	Axon	Normal	Normal	12	•	•	•	Na^+
DUMETi	Soma	Normal	Normal	12	74	4·4	15	Na^+–Ca^{2+}
DUMETi	Soma	Normal	TEA	2	65	7·0	0	Na^+–Ca^{2+}
DUMETi	Soma	Axotomy	Normal	2	74	3·0	12	Na^+
FETi	Axon	Normal	Normal	10	•	•	•	Na^+
FETi	Soma	Normal	Normal	17		Non-spiking		—
FETi	Soma	Normal	TEA	10	61	10·2	5	Na^+–Ca^{2+}
FETi	Soma	Colchicine or axotomy	Normal	7	59	1·3	12	Na^+

All resting potentials for FETi and DUMETi somata ranged from −40 to −60 mV and for DCMD axon was −70 mV.
• Intracellular data on these axon spikes were not obtained.

They concluded that the inward current of the soma spike of DUM cells is carried predominantly by Na^+. However, they only demonstrated that Na^+ is necessary for the soma spike, since perfusion with Na^+-free saline abolished the action potential. No attempt was made to block Ca^{2+} channels and thus they did not address the issue of the necessity for Ca^{2+}. We find that blocking Ca^{2+} channels with Co^{2+} or La^{3+} also abolishes the soma spike. Thus, their conclusion seems unwarranted because they never clearly eliminated a role for Ca^{2+}.

Electrical properties of FETi soma

The soma of FETi normally does not generate action potentials. Only attenuated axon spikes (mean amplitude 21·1 mV at 22 °C, but highly temperature dependent) are recorded in the soma, having been electrotonically propagated from the axon. Axon spikes evoked by intracellular injection by depolarizing current into the soma have a similar amplitude to axon spikes evoked by antidromic stimulation, but a shorter duration (mean of 1·2 compared to 2·9 ms), and a much larger afterpotential (mean 20·4 compared to 2·3 mV). The phenomena could be explained by an active response of the soma membrane, if depolarization of the soma increased the outward K^+ current (delayed rectification). We show that delayed rectification, occurring at depolarizations of 15–20 mV from resting potential can reduce the mean input resistance of FETi from 3·3 to 2·0 MΩ.

When some of the outward K^+ current is blocked by TEA, the soma of FETi is capable of generating overshooting action potentials. This phenomenon was first described by Pitman (1975*b*) in the soma of the fast coxal depressor (D_f) motorneurone of the cockroach, who reported that the predominant inward current of this TEA-induced soma spike was carried by Ca^{2+}; perfusion with Na^+-free or TTX had little effect while Mn^+ abolished the action potential. We find that the inward current of the TEA-induced soma spike in FETi of the locust is carried by both Na^+ and Ca^{2+}; removal of Na^+, addition of TTX or addition of Co^{2+} (or La^{3+}) abolished the action potential. There are several possible explanations for the difference between Pitman's findings of a TEA-induced Ca^{2+} spike in D_f of the cockroach and our finding of a TEA-induced Na^+–Ca^{2+} spike in FETi of the locust. There might be a difference in the membrane properties of motorneurones between the two species. Alternatively, there might be a difference between types of motorneurones even within the same species. In this regard, it would be of interest to examine the effects of TEA on the somata of many different motorneurones and interneurones in the metathoracic ganglion of the locust and the cockroach.

Effects of axotomy or colchicine treatment on FETi

Axotomy or treatment with colchicine converts the non-spiking soma of FETi within 4 days into a spiking soma, capable of generating overshooting action potentials. This phenomenon was first described by Pitman *et al.* (1972) in the soma of the D_f motorneurone of the cockroach. Pitman (1975*a*) concluded that Na^+ was the major carrier of inward current of the action potential induced by colchicine. We similarly find that Na^+ is the major carrier of inward current of the action potential induced by axotomy or colchicine treatment in the soma of FETi, since it is blocked only by removal of Na^+.

Since an axotomy-induced soma spike had a significantly shorter duration, a larger afterpotential, and a different ionic dependence than a soma spike produced by addition of TEA (Table 2), we conclude that axotomy is not simply reducing the voltage dependent outward K^+ current. Since R_{in} decreases with axotomy, there is unlikely to be a decrease in the resting K^+ current. This suggests the possibility of an increased inward current in the soma of the axotomized or colchicine-treated FETi. If the voltage dependent divalent cation channels changed, then we might expect a change in either the amplitude, duration, threshold, or repolarization potential of the Ba^{2+} soma spike. However, the Ba^{2+} action potential (in Na^+-free plus Ba^{2+}) appears normal in all four of these parameters.

The axotomy or colchicine-induced Na^+ soma spikes in FETi appear to be the result of an increase in monovalent inward current. One possibility is that there is a significant increase in the concentration gradient across the membrane, thus increasing the Na^+ reversal potential. However, this would require a separate explanation for the decreased R_{in} and is not consistent with the effects of axotomy on DUMETi (see below). The most likely explanation for the increased Na^+ inward current is an increased number of active Na^+ channels, either by addition of new channels or modification or redistribution of existing channels. The possibility of new protein synthesis is suggested by the observation that axotomy or colchicine treatment both induce formation of perinuclear RNA rings (Cohen & Jacklet, 1965; Pitman *et al.* 1972). We suggest that the increase in inward current after both of these treatments may be due to new synthesis and/or insertion of Na^+ channels, a possibility that awaits further investigation.

Effects of axotomy on DUMETi

Axotomy had little effect on the duration, amplitude, and afterpotential of the soma spike in DUMETi, suggesting that there has not been a change in Na^+ concentration gradient across the soma. However, in the normal DUMETi, Na^+ and Ca^{2+} inward currents are both necessary for the generation of a soma spike, whereas in the axotomized DUMETi, Na^+ alone is sufficient for the generation of a soma spike. We also find evidence for an increase in excitability in the neuropil of DUMETi. The region of membrane about the branch point in which spikes often fail in the normal animal is converted to a fully active region of membrane in which spikes never fall. Both of these changes in excitability can be explained by an increased number of Na^+ channels, similar to the suggested effects of axotomy on the soma of FETi.

Spiking vs. *non-spiking somata: an hypothesis*

Why is the soma of DUMETi normally spiking while the soma of FETi is non-spiking? The somata of both FETi and DUMETi normally contain both Na^+ and Ca^{2+} inward current channels. The soma spike in FETi induced by TEA is a mixed-dependency Na^+–Ca^{2+} action potential just as is the normal soma spike in DUMETi. Although the normal soma spike in DUMETi is shorter in duration than the TEA-induced soma spike of FETi, much of this difference in duration is reduced by the effects of TEA on the soma spike of DUMETi (Table 2). Thus, when some of the outward current is blocked by TEA, the inward current channels of both somata can generate similar action potentials. Furthermore, when some of the outward current is

blocked by Ba^{2+}, the divalent cation channels of both somata can generate similar Ba^{2+} action potentials. This suggests that there is no great difference between the inward current channels of DUMETi and those of FETi. The reason that DUMETi but not FETi has a soma spike may be that the former has a weaker outward K^+ current in its soma than the latter, which allows the somatic membrane potential to escape into the regenerative portion of the action potential cycle under the influence of the inward Na^+ and Ca^{2+} currents.

What is the functional significance of spiking somata? The bilaterally paired motorneurones and interganglionic interneurones, representing the majority of neurones in the segmental ganglia, all have somata that are non-spiking (as far as is known). Spiking somata have only been found in the special group of neurones called dorsal unpaired median (DUM) neurones. The DUM neurones appear to have an innervating neurosecretory role rather than conventional motor function (Hoyle *et al.* 1974). DUMETi can modulate an intrinsic myogenic rhythm in the ETi muscle (Hoyle & O'Shea, 1974; Hoyle, 1974; Evans & O'Shea, 1978), and it can modulate the electrical and mechanical effect produced by normal motorneurones innervating this muscle (Evans & O'Shea, 1977). The modulatory effects of DUMETi can be mimicked by the application of low concentrations of octopamine to the isolated muscle (Hoyle, 1974; Evans & O'Shea, 1977, 1978), and the soma of DUMETi has been shown to contain octopamine (Evans & O'Shea, 1977, 1978). Do the spiking somata of the DUM neurones somehow reflect this specialized role? One possible function of the soma spike in DUM neurones is for signalling to the soma, possibly by Ca^{2+} influx, the activity of the neurone for long-term metabolic functions. One observation argues against this suggestion. At the temperature at which the animals are raised (32 °C), the synaptically driven axons spikes do not always evoke soma spikes as they do at room temperature (Heitler & Goodman, 1978). Thus soma spikes may not always occur during neuronal activity in the intact animal at its environmental temperature. A second possibility is that the somata of these neurones are themselves involved in secretory release. No evidence yet argues for or against this suggestion. A third possibility is that the soma spike is not in itself important, but rather is an indication of some difference in the axon or processes of the neurone. We suggest that the soma of DUMETi is able to spike because it has a decreased outward current relative to the soma of FETi. There might also be decreased outward current and thus longer duration action potentials in the axon of DUMETi as compared to that of FETi or most neurones. The soma spike may thus simply reflect a longer duration action potential at the site of synaptic release. In this regard, it is possible that longer duration action potentials are required when conventional synapses are not formed but rather amines are released as synaptic modulators into areas of tissue rather than across synaptic gaps. A recent survey of the electrophysiology of neurosecretory neurones in a wide variety of animals within many phyla showed that 'in all cases the duration of the action potential is 2–10 or more times longer than that of comparable non-neurosecretory neurones' (Mason & Bern, 1977). It is possible that a decrease in outward current is responsible for the longer duration action potentials in many of these cases.

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