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CALCIUM SPIKES AND CALCIUM PLATEAUX EVOKED BY DIFFERENTIAL POLARIZATION IN DENDRITES OF TURTLE MOTONEURONES *IN VITRO*

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SUMMARY

1. The ability of dendrites in turtle motoneurones to support calcium spikes and calcium plateaux was investigated using differential polarization by applied electric fields.

2. Electric fields were generated by passing current through transverse slices of the turtle spinal cord between two plate electrodes. The linear extracellular voltage gradient generated by the field implied that the tissue was ohmic and homogeneous.

3. The transmembrane potential at the cell body of motoneurones was measured as the voltage difference between an intracellular and an extracellular microelectrode.

4. In normal medium an applied field induced synaptic activity as well as intrinsic polarization of motoneurones. Synaptic activity was suppressed by tetrodotoxin (TTX, 1 μM).

5. In the presence of TTX and tetraethylammonium (TEA, 1–5 mM), applied fields evoked multicomponent Ca^{2+} spikes in both the soma-hyperpolarizing and soma-depolarizing direction of the field. The different components of Ca^{2+} spikes were discrete and additive. High amplitude components had higher threshold and faster time course and were followed by larger after-hyperpolarizations, than low amplitude components. The frequency of field-evoked regenerative responses was relatively insensitive to somatic bias current.

6. TTX-resistant Ca^{2+}-mediated plateau potentials promoted by apamin were evoked by differential polarization in both the soma-depolarizing and soma-hyperpolarizing direction.

7. It is concluded that Ca^{2+} channels responsible for Ca^{2+} spikes and Ca^{2+} plateaux are present in dendrites of spinal motoneurones of the turtle.

INTRODUCTION

In vertebrate neurones, voltage-sensitive ion channels determine the cell-specific response patterns evoked by intracellularly applied current pulses (Llinás, 1988). How these active membrane properties are distributed in the membrane of the cell body and dendrites is mostly unknown. In cerebellar Purkinje cells (Llinás & Nicholson, 1971; Llinás & Sugimori, 1980; Hounsgaard & Midtgaard, 1988) and pyramidal cells from cortex (Huguenard, Hamill & Prince, 1989) and hippocampus

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(Benardo, Masukawa & Prince, 1982), the membrane properties of dendrites have been characterized in detail from intradendritic recordings. In a few other cell types a dendritic location of certain ion conductances has been deduced from data obtained in somatic recordings (Llinás, 1988). For most nerve cells, however, the question remains unsettled. Information about local electrophysiological properties of dendrites is lacking because intradendritic recordings cannot be obtained and because the origin of active responses observed in somatic recordings cannot be decided. Spinal motoneurones are prominent members of this latter group of nerve cells.

Two types of Ca^{2+} conductances and several types of K^+ conductances are known to contribute to the active response properties monitored in cell body recordings from the spinal motoneurones of several vertebrate species (Barrett & Barrett, 1976; Schwindt & Crill, 1984; Hounsgaard & Mintz, 1988). The spatial distribution of the conductances has not been determined with any certainty, but extensive voltage clamp experiments in the cat *in vivo* were based on, and partly supported, the assumption that channels were located mainly in the cell body and proximal dendrites (Schwindt & Crill, 1984), although a more distal presence could not be entirely ruled out (Barrett & Crill, 1980). Other studies indicate that dendrites of motoneurones may support regenerative responses (Burke & Rudomin, 1977; Walton & Fulton, 1986; Fujita, 1989). In addition, the plateau potentials described in motoneurones (Hounsgaard, Hultborn, Jespersen & Kiehn, 1988*a*; Hounsgaard & Kiehn, 1989) were foreseen and predicted to have a dendritic location in theoretical studies (Gutman 1971, 1991).

In the present study we use Ca^{2+} spikes and Ca^{2+} plateaux, promoted by TEA and apamin respectively, to probe distal dendrites for the presence of Ca^{2+} channels. Localized depolarization was achieved by differential polarization of the membrane potential by applied electric fields during intracellular recordings from cell bodies of motoneurones in an *in vitro* preparation of the turtle spinal cord. We find that Ca^{2+} spikes and Ca^{2+} plateaux can be initiated in distal dendrites as well as near to the cell body. Our experiments also suggest that some types of K^+ channels are present in distal dendrites. These findings indicate that active membrane properties may be intimately involved in synaptic integration in motoneurones. Some of the results have been published in abstract form (Hounsgaard & Kiehn, 1990*a*, *b*).

METHODS

Preparation. Transverse sections of the lumbar spinal cord were obtained as described before (Hounsgaard, Kiehn & Mintz, 1988*b*; Hounsgaard & Kiehn, 1989) from turtles (*Pseudemys scripta elegans*) deeply anaesthetized by 100 mg pentobarbitone injected intraperitonally.

Field stimulation. For experiments a section of the cord, 2–3 mm thick, glued on end to a piece of filter paper, was placed in the recording chamber between two plate electrodes (Fig. 1*A*). The plate electrodes consisted of tables of sintered silver–silver chloride with a surface area of 12 mm^2. These electrodes carried at least 10 mA for 3 s without signs of polarization. Current for field stimulation was delivered by an isolation unit, model BSI-1 from BAK Electronic Inc. or, in later experiments, by a homemade equivalent powered by a 500 V battery package. The characteristics of the electric field induced in the spinal cord preparation were investigated in four separate experiments. The voltage gradient in the tissue was 10–20 mV mm^{-1} mA^{-1} 200–300 μm below the surface in the direction of the field. The voltage gradient was proportional to the current applied and there was no significant difference between the gradient in grey and white matter. The voltage gradient vertically in the tissue 200–300 μm below the surface was less than 10% of the field

between the electrodes in the transverse plane. Thus, when measured with an electrode separation of at least 100 μm and more than 50 μm from a surface, the tissue was ohmic and homogeneous in response to applied fields. These findings are in full agreement with the results obtained in the isolated cerebellum of the turtle (Chan & Nicholson, 1986; Chan, Hounsgaard & Nicholson, 1988).

Recording of transmembrane potential. Transmembrane potentials from the cell body of motoneurones were obtained as described in detail for Purkinje cells (Chan *et al.* 1988). Two electrodes were positioned in the motor nucleus of the ventral horn. When one, filled with 2 M potassium acetate, penetrated a neurone, the other, broken and filled with 1 M NaCl, was adjusted to the same depth below the cut surface. The potentials recorded by the two electrodes were fed to a unity gain differential amplifier. This arrangement is illustrated diagramatically in Fig. 1*B*. Turtle motoneurones extend dendrites radially in the transverse plane from the cell body in the motor nucleus. Thus, the recording site in the cell body is near the indifference point for the field-induced polarization of the membrane potential (arrow in Fig. 1*B*) while maximal polarization is induced in terminal dendrites extending towards the two field electrodes. The curves in Fig. 1*B* indicate the spatial relation between the extracellular potential, V_e, and the transmembrane potential, V_m, for the two directions of polarizing current. In the example shown the recording site in the cell body polarizes in phase with the medially projecting dendrites and out of phase with the laterally projecting dendrites. In this paper, the direction of polarizing current that cause soma-depolarization is termed S^+ and the opposite direction causing soma-hyperpolarization is termed S^-.

In normal medium, electric fields induced a mixture of intrinsic and synaptic responses in motoneurones. As illustrated in Fig. 1*C* for a soma-depolarizing field, S^+, and in Fig. 1*D* for a soma-hyperpolarizing field, S^-, the synaptic and regenerative components were suppressed by tetrodotoxin (TTX). In other experiments the early depolarizing transient during S^- stimuli was suppressed by a combination of 10 μM 6-cyano-7-nitroquinoxaline (CNQX), 1 μM bicuculline, 1 μM 2-amino-5-phosphonovaleric acid (APV), 0·1 mM picrotoxin and 1 μM strychnine and therefore considered synaptic in nature.

At the end of each recording session the intracellular electrode was withdrawn to an extracellular position. The extracellular voltage difference between the two recording electrodes in response to a field stimulus of 10 mA was recorded. In cases where the voltage difference exceeded 5 mV the transmembrane potential was corrected off-line (Chan *et al.* 1988). Data stored on tape included the extracellular potential and the voltage difference between the intracellular and extracellular electrode.

Solutions. Normal medium contained (mM): 120 NaCl, 5 KCl, 15 $NaHCO_3$, 20 glucose, 2 $MgCl_2$ and 3 $CaCl_2$. The solution was saturated with 98% O_2–2% CO_2 to obtain a pH of 7·6 in the recording chamber. Drugs and chemicals were added to this medium as indicated in the text. Tetrodotoxin, tetraethylamonium, apamin, bicuculline and strychnine were obtained from Sigma, CNQX and APV from Tocris Neuramin.

Database. The results are based on records from sixty cells identified as motoneurones on the basis of their characteristic firing pattern and spike configuration (Hounsgaard *et al.* 1988*b*). These cells had a resting membrane potential of at least −60 mV and spike heights of more than 85 mV.

RESULTS

The general effects of differential polarization on the transmembrane potential measured at the soma were very similar for the population of sixty lumbar motoneurones included in the present study. This is in agreement with the uniformity of dendritic arbors in turtle motoneurones (Rugirok, Crowe & Ten Donkelaar, 1985) and suggests comparable uniformity with respect to active response properties. As with current pulses (Hounsgaard & Kiehn, 1985; Hounsgaard *et al.* 1988*b*), field stimulation did not generate regenerative responses in the presence of TTX unless the total outward current was reduced. In the experiments to be described here we have used TEA to promote Ca^{2+} spikes and apamin to promote Ca^{2+} plateaux (Hounsgaard & Mintz, 1988).

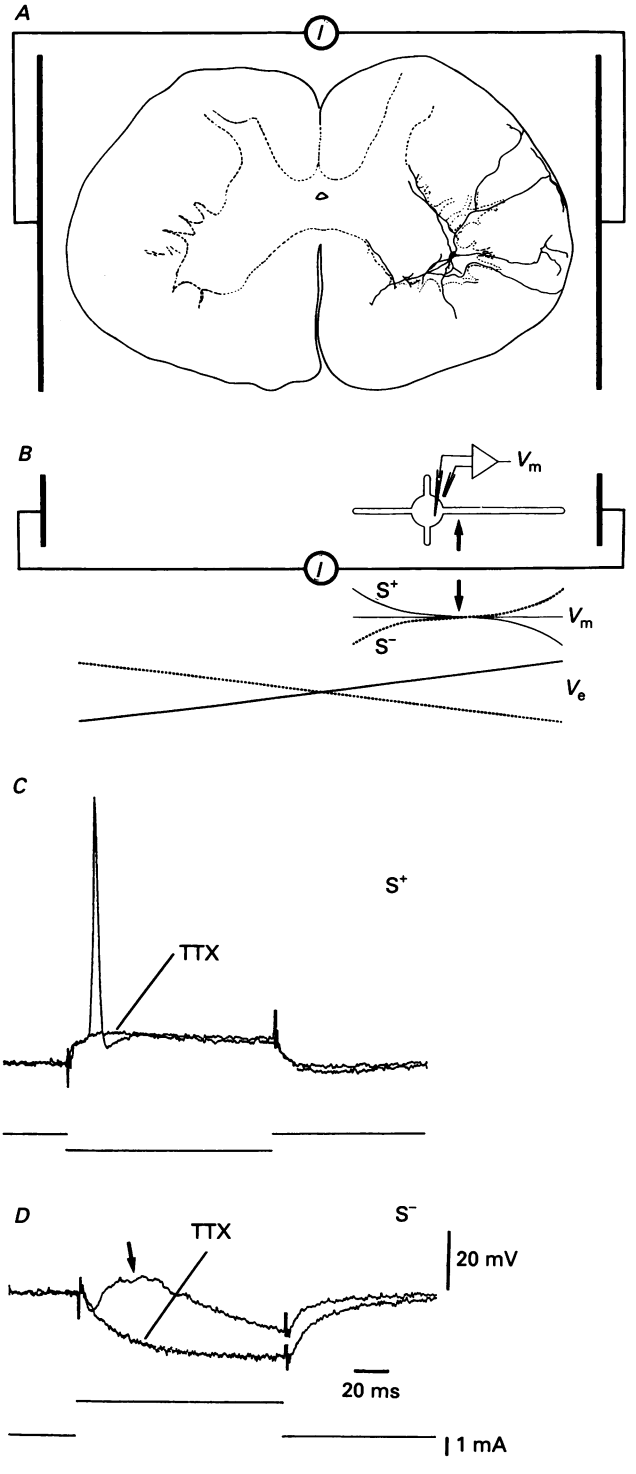

Fig. 1. For legend see facing page.

Field-induced Ca^{2+} spikes

In the presence of 1 μM TTX and 1–5 mM TEA, a train of Ca^{2+} spikes was evoked during a depolarizing current pulse of sufficient amplitude, while no regenerative responses occurred during hyperpolarizing pulses (Fig. 2*A*). A different picture emerged in response to externally applied electrical fields. Figure 2*B* shows representative recordings during application of soma-depolarizing (S^+) and a soma-hyperpolarizing (S^-) fields. Unlike intracellularly injected current, the applied fields clearly evoked regenerative responses with a wide range of amplitudes. These responses occurred both with the soma-hyperpolarizing directions of the field although spikes with amplitudes like the spikes evoked by intracellular current pulses were more readily observed with soma-depolarizing fields. The TTX-resistant field-induced regenerative responses were blocked when Ca^{2+} was replaced by Co^{2+} in the medium. The discrete nature of the variations in amplitude of the regenerative responses evoked by applied fields is illustrated in more detail in Fig. 2*C* and *D*. Figure 2*C* compares regenerative responses in expanded sweeps from records like those in Fig. 2*A* and *B*. Although the spikes evoked by injected current (Fig. 2*Ca*) tended to have longer durations than the full spike evoked by a soma-depolarizing field (Fig. 2*Cb*) there was no measurable difference in time to peak (measured from the spike threshold). Unlike with current injection, however, the field-evoked spikes were always composed of subcomponents, as evidenced by inflexion points on the rising phase. Figure 2*D* shows more directly that the full spike evoked by a field stimulus is triggered from a subcomponent in an all-or-none manner. In addition it shows that the dissociation between the spike components can be obliterated by a depolarizing holding current through the recording electrode. From Fig. 2*Cb* and *c* it is also evident that the field response is not a smoothly oscillating potential but is composed of several additive unitary events (arrows). Since the discrete events closely resemble the all-or-none spike components described for dendrites in Purkinje cells (Llinás & Nicholson, 1971; Llinás & Hess, 1976) and in immature motoneurones (Walton & Fulton, 1986), we use the word spike rather than subthreshold oscillations throughout the paper.

The relation between the strength of the applied field and the evoked regenerative responses is illustrated in Fig. 3 with the responses to soma-depolarizing fields in the left panel and to soma-hyperpolarizing fields in the right panel.

In each sweep of the left panel it is possible to distinguish two or three classes of responses in terms of peak amplitudes. It is also apparent that there is a direct relation between the level of the peak depolarization for each regenerative response and the amplitude of the after-hyperpolarization. Likewise the high threshold components of the spikes are faster than low threshold components. The main

Fig. 1. Differential polarization and recording of membrane potential in motoneurones. *A*, a voltage gradient is generated by passing current across the preparation between two plate electrodes. *B*, arrangement for measurement of transmembrane potential at the soma. The spatial profiles of transmembrane potential, V_m, and extracellular potential, V_e, for two polarities of stimulus current are indicated below. Note that the recording site is eccentric with respect to the indifference point for the transmembrane potential (arrows). This defines a soma-hyperpolarizing, S^-, and a soma-depolarizing, S^+, direction of the field. *C* and *D* show records of transmembrane potential during an S^+ field (*C*) and an S^- field (*D*) before and after an application of 1 μM TTX.

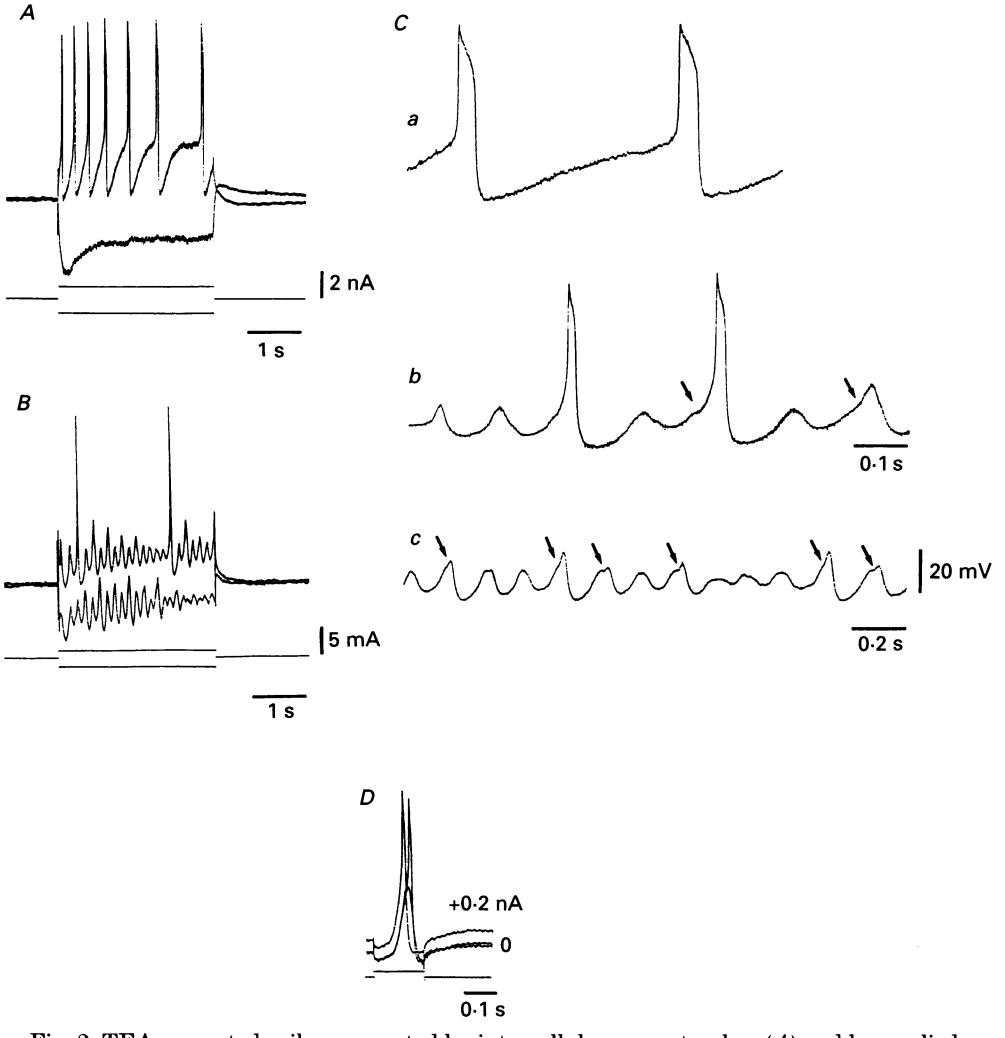

Fig. 2. TEA promoted spikes generated by intracellular current pulses (*A*) and by applied electric fields (*B–D*) in the presence of TTX. Intracellular current generated a train of Ca^{2+} spikes with gradual adaptation and spike broadening during depolarizing pulses and no regenerative responses during hyperpolarizing currents (*A*). With electric fields a complex pattern of regenerative responses are observed with both soma-hyperpolarizing (S^-) and soma-depolarizing (S^+) stimuli (*B*). Traces of regenerative responses in high sweep speed are shown in *C*, evoked by intracellular applied depolarizing current (*a*), a soma-depolarizing field (*b*) and a soma-hyperpolarizing field (*c*). Note that multicomponent regenerative responses (arrows) only occur with differential polarization (*b* and *c*). In *D* the regenerative response evoked by soma-hyperpolarizing field consists of two discrete components at rest that fuse and occur with shorter latency with depolarizing bias current of +0·2 nA. 1 μM TTX, 2 mM TEA.

characteristics of the regenerative response evoked during soma-hyperpolarizing fields are similar. Note that the increase in amplitude of responses with strength of soma-hyperpolarizing fields is mainly due to summation of the separate components

of the responses seen at low stimulus strength (Fig. 3*B*) as their overlap increases with higher stimulus strength.

The experiments illustrated in Figs 2 and 3 show that the field-evoked Ca^{2+} spikes in the presence of TTX and TEA are composed of distinct subcomponents. The

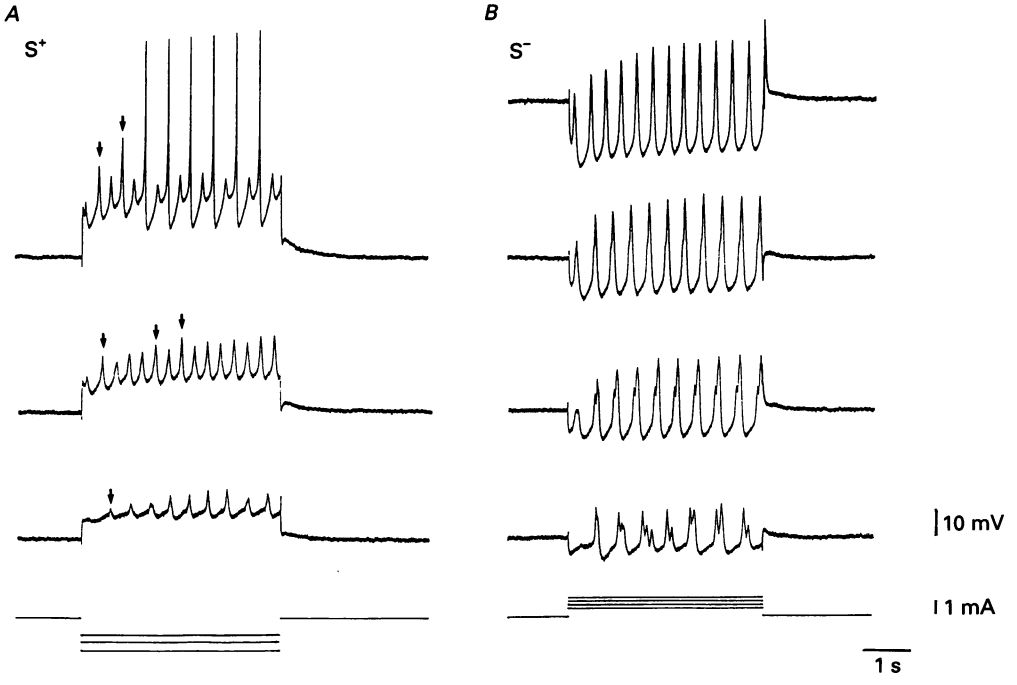

Fig. 3. Effect of stimulus intensity on regenerative Ca^{2+} responses evoked by soma-depolarizing (*A*) and soma-hyperpolarizing electric fields (*B*). The amplitude and frequency of regenerative responses increase and the duration decreases with increasing intensity of the field. Note the discrete nature of regenerative responses. Note also that synchronization of elements is better in the S^+ direction of the field than in the S^- direction. 1 μM TTX, 2 mM TEA.

additive nature of the spike components suggest that they are local responses with multiple sites of origin. This conclusion was further supported by the results obtained with a field stimulus of constant amplitude applied at different levels of holding current injected through the recording electrode (Fig. 4). In Fig. 4*A* the middle sweep was obtained without holding current. The same pulse applied with a depolarizing holding current of +0·5 nA increased the number of high amplitude spikes while a hyperpolarizing holding current of −0·5 nA decreased the number of high amplitude spikes. It is important to note, however, that the frequency of regenerative responses was unaffected by the level of holding current. Note also that each high amplitude spike is followed by a deep after-hyperpolarization that apparently prevents high amplitude components in the first spike that follows.

With soma-hyperpolarizing field stimuli, depolarizing holding current also facilitated generation of high amplitude regenerative responses. This was mediated,

at least in part, by an increased overlap of the subcomponents of the responses at rest (Fig. 4*B*).

The effects of holding current on field-evoked regenerative responses is in agreement with the suggestion that the sites of origin for the different components

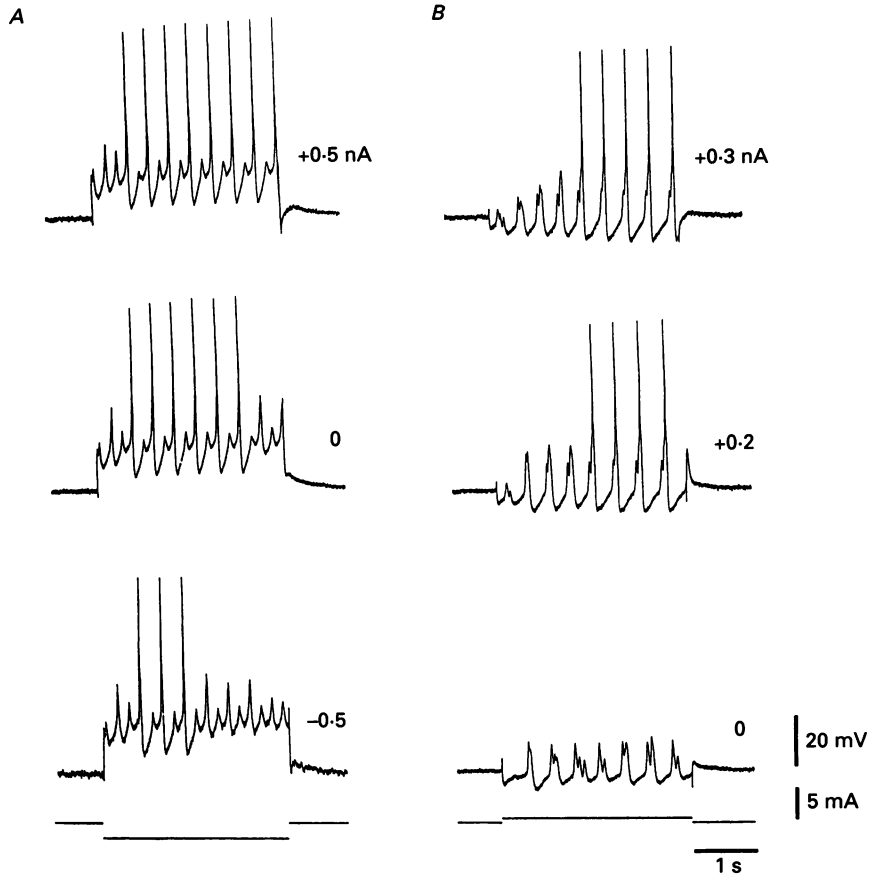

Fig. 4. Effect of intracellular bias current on regenerative calcium responses evoked by a soma-depolarizing field (*A*) and a soma-hyperpolarizing field (*B*). The number of high amplitude elements increases with depolarizing holding current and decreases with hyperpolarizing holding current, with only weak effects on the frequency of regenerative responses. 1 μM TTX, 2 mM TEA, holding current indicated with each trace.

of the regenerative responses are compartmentally separated, with the large amplitude components being generated close to the recording site and the smallest components more distant.

Field-induced Ca^{2+} plateaux

In turtle motoneurones, apamin (1–10 μM) promotes a Ca^{2+}-mediated, nifedipine-sensitive plateau potential (Hounsgaard & Mintz, 1988) but no Ca^{2+} spikes. The experiments illustrated in Fig. 5 show that applied electric fields in both the soma-

depolarizing and the soma-hyperpolarizing directions can evoke plateau potentials in the presence of apamin. (Fig. 5*A* and *B*, lower sweeps). Of particular importance is the finding that the plateau response is recruited *during* the soma-hyperpolarizing stimulus and yet reaches a sufficiently depolarized level for supporting spike

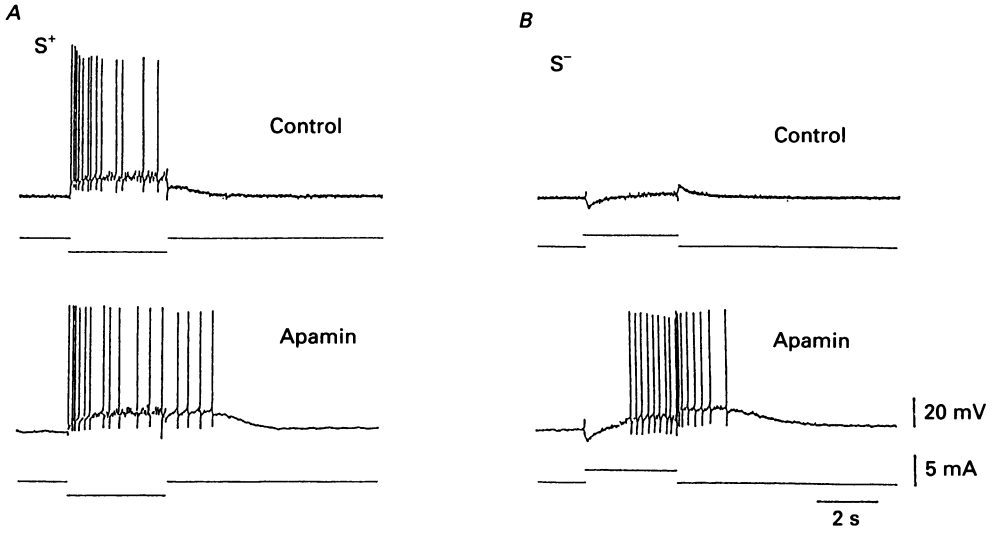

Fig. 5. Response to a soma-depolarizing field (*A*) and a soma-hyperpolarizing field (*B*) before and after application of 1 μM apamin. The lower sweeps in *A* and *B* show that apamin-promoted plateau potentials are recruited during field stimuli in both the soma-depolarizing (S^+) and the soma-hyperpolarizing (S^-) directions and outlast stimuli by several seconds.

generation (Fig. 5*B*, lower sweep). As with a soma-depolarizing field (lower sweep in Fig. 5*A*) the plateau remains activated for some seconds after the stimulus. These basic observations suggested that plateau potentials could be generated in dendrites of turtle motoneurones. This was tested in more detail as illustrated by the experiments in Figs 6 and 7.

Figure 6 serves to compare the characteristics of the plateau potential activated and terminated by intracellularly injected current pulses (Fig. 6*Aa* and *Ba*) and by differential polarization (Fig. 6*Ab*, *Ac*, *Bb* and *Bc*). The experiments were conducted in normal medium (Fig 6*Aa–c*) and then repeated in the presence of TTX (Fig. 6*Ba–c*). In six cells, in which apamin- or 5-HT-induced plateaux could be evoked by depolarizing current pulses, a plateau with similar amplitude and duration could also be evoked by soma-hyperpolarizing and soma-depolarizing electric fields. Plateau potentials were readily terminated by hyperpolarizing current pulses through the recording electrode as in Fig. 6*Aa* and 6*Ba*. This was rarely possible with depolarizing fields (Fig. 6*Ab*) and only in a narrow range of holding current and field intensity. Hyperpolarizing fields were never able to terminate plateau potentials (Fig. 6*Ac* and *Bc*).

The plateau potentials showed the same sensitivity to membrane potential when evoked with differentially polarizing electric fields, as previously found with injected

current pulses (Hounsgaard & Kiehn, 1985, 1989; Hounsgaard & Mintz, 1988). The experiments illustrated in Fig. 7 were conducted in the presence of TTX and apamin. In Fig. 7*A* a sustained plateau was activated by a soma-hyperpolarizing field stimulation and terminated by a soma-depolarizing field stimulation when a

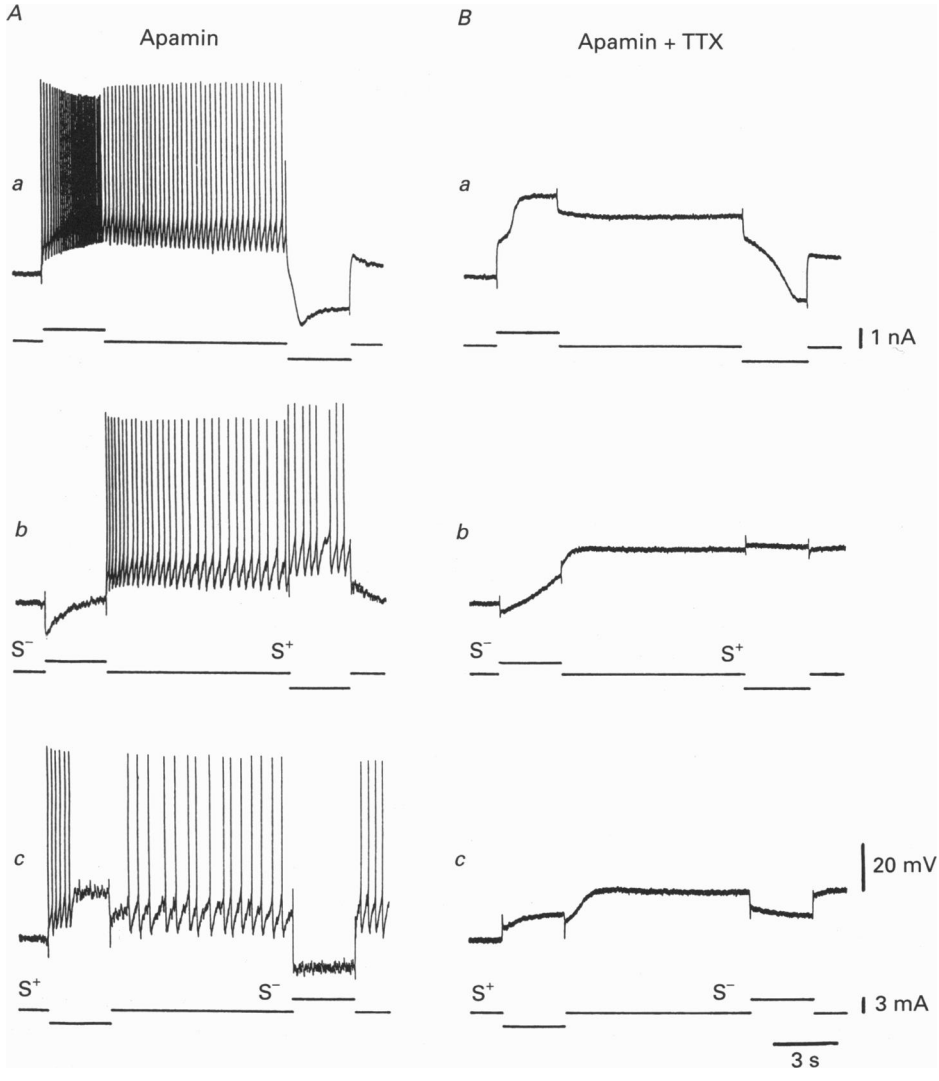

Fig. 6. Apamin promoted bistability before (*A*) and after (*B*) application of TTX. *Aa* and *Ba*, plateau potentials activated by depolarizing current pulses and terminated by hyperpolarizing current pulses. *Ab* and *Bb*, plateau potentials activated by soma-hyperpolarizing fields (S^-) and terminated by soma-depolarizing fields (S^+). *Ab* and *Bc*, plateau potential activated by soma-depolarizing field (*Ac*). In *Ac*, *Bb* and *Bc* plateaux could not be terminated by applied electric fields. 1 μM TTX, 1 μM apamin.

constant current of 0·2 nA was passed through the recording electrode. Without holding current (middle trace in Fig. 7*A*) the plateau was activated during the soma-hyperpolarizing stimulus, but decayed during the first few seconds after the stimulus.

Finally with a −0·2 nA bias current (lower trace) no signs of plateaux were observed with either polarity of the applied field. After application of nifedipine, the voltage-sensitive plateau potentials evoked by field stimulation were greatly reduced as shown in Fig. 7*B*.

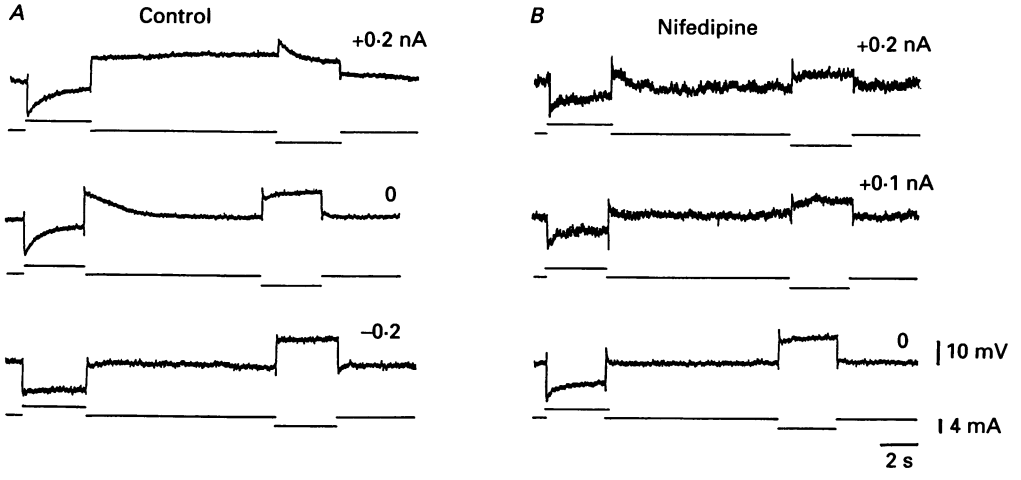

Fig. 7. Effect of bias current (*A*) and nifedipine (*B*) on field-induced plateaux in the presence of TTX and apamin. *A*, with a depolarizing holding current of 0·2 nA a soma-hyperpolarizing field stimulus (S^-) induces a plateau terminated by a soma-depolarizing field stimulus (S^+) (upper sweep). At rest (0 nA) the same sequence of stimuli only induce a transient plateau that decays well before the soma-depolarizing field (middle trace). With a hyperpolarizing bias current of 0·2 nA the sequence of field stimuli induce no obvious active responses (lower sweep). *B*, the field-induced plateaux are reduced by nifedipine at all holding currents. 1 μM TTX, 1 μM apamin, 5 μM nifedipine.

DISCUSSION

The main finding in the present paper is that Ca^{2+} spikes and Ca^{2+} plateaux can be generated by dendrites in spinal motoneurones.

The results obtained are based on the differential polarization technique developed and evaluated for use in combination with measurements of transmembrane potentials in cerebellar Purkinje cells (Chan *et al.* 1988; Chan, Housgaard & Mitgaard, 1989; Lopez, Chan, Okada & Nicholson, 1991). With this technique the membrane potential can be differentially polarized in cells with an extension in the direction of the field. The magnitude and distribution of the polarization in neurones is determined by the cable structure and internal resistance of the cell (Tranchina & Nicholson, 1986) and in cells with active membrane properties additionally by the kind, density and distribution of voltage-sensitive ion channels. Since none of these parameters are known quantitatively we have aimed only at qualitative assessments. This limitation is further reinforced by the fact that the transmembrane potentials in the present study were recorded solely from the soma.

In turtle motoneurones dendrites radiate in the transverse plane of the spinal cord and terminate a dense subpial or marginal plexus (Ruigrok *et al.* 1985). Because of the special significance of bends in focusing polarization in cables (Tranchina & Nicholson, 1986; Chan *et al.* 1989), an applied field is expected to have a particularly

strong effect on the membrane potential in terminal dendrites. We suggest that the field-evoked Ca^{2+} spikes originate in distal dendrites and spread towards the soma. First, the TEA-promoted Ca^{2+} spikes evoked during application of electric fields were elicited with both soma-hyperpolarizing and soma-depolarizing fields. This shows that the activity was not generated exclusively by Ca^{2+} channels in soma-near membranes. Second, the frequency of the spikes evoked by electric fields was relatively immune to changes in somatic membrane potential induced by holding current, but responded readily to changes in field intensity. This shows that the field-evoked spikes originate from sites electronically remote from the cell body. The existence of remote sites of generation is also supported by the fact that the regenerative responses with the lowest threshold were of low amplitude and slow rates of rise and fall in somatic recordings. We also conclude that there must be multiple, spatially separated generation sites for regenerative Ca^{2+} responses since the different components are summated electrotonically. Generation sites close to the cell body are evidenced by the increased spike height and shorter duration observed with more intense depolarization of the soma-near compartments. A simple way of explaining the results on TEA-promoted Ca^{2+} spikes is therefore to assume that the Ca^{2+} channels involved are present throughout the dendritic tree and possibly also in the cell body. The latter is suggested by the fact that maximally high and fast spikes were observed only in combination with soma-depolarization beyond the resting membrane potential, be it by injected current, applied electric field or electrotonic potentials.

The density of Ca^{2+} channels is probably low since spike propagation required widespread depolarization. It also seems necessary to assume that the TEA-sensitive K^+ channels that normally prevent regenerative Ca^{2+} responses are widely distributed for them to influence all the potential sites for spike generation. The discrete nature of spike components suggests they have relatively stable and well-defined compartments of origin. We suggest that such compartments could be the cables connecting dendritic branch points.

The most compelling evidence for the presence of generation sites for Ca^{2+}-mediated plateau potentials in the dendrites of turtle motoneurones is the finding that plateaux were readily activated by a soma-hyperpolarizing electric field. Apart from being present in distal dendrites, it is not clear how the dihydropyridine-sensitive Ca^{2+} channels responsible for plateau potentials are distributed, mainly because subcomponents like those seen with field-evoked Ca^{2+} spikes have not been observed with field-evoked plateaux.

Our experiments have not produced any evidence against a homogeneous distribution of spike- and plateau-generating Ca^{2+} channels in somatic and dendritic membranes in motoneurones. With the limited spatial resolution of differential polarization, however, it would be possible for various forms of channel clustering to go unnoticed in our experiments.

In Purkinje cells and hippocampal pyramidal cells voltage-sensitive ion channels in dendrites directly contribute to the intrinsic membrane properties (Llinás & Sugimori, 1980; Benardo *et al.* 1982) and create a link to intracellular signalling via changes in the cytoplasmic concentration of Ca^{2+} (Sugimori & Llinás, 1990; Jaffe, Johnston, Lasser-Ross, Lisman, Miyakawa & Ross, 1992). The presence of voltage-

sensitive channels in dendrites of motoneurones suggests a similar role in cells that previously were thought to have passive dendrites. It is possible that the channels hinder attenuation of distally generated synaptic potentials and, in addition, provide the means for local synaptic interactions (Gutman, 1991; Hounsgaard & Midtgaard, 1989; Midtgaard & Hounsgaard, 1989).

Many voltage-gated channels are modulated by transmitter-regulated second messengers (Hille, 1991) and the intrinsic properties of many neurones can be shifted over a wide range by modulatory transmitters (Nicoll, 1988; McCormick & Pape, 1990). In motoneurones serotonin is known to affect the intrinsic properties by modulating several different types of ion channels (Hounsgaard & Kiehn, 1989; Berger & Takahashi, 1990; Takahashi & Berger, 1990; Kiehn 1991*a*). In turtle motoneurones serotonergic terminals are located both around the cell bodies and along the dendrites (Kiehn *et al.* 1992). This suggests that different compartments of the cells can be modulated independently. These and other possible consequences of dendritically located voltage-gated ion channels in motoneurones are yet to be investigated. So far the established role for the channels generating Ca^{2+} spikes is to mediate Ca^{2+} influx during action potentials and thereby activate the current underlying the long-lasting after-hyperpolarization (Barrett & Barrett, 1976; Hounsgaard *et al.* 1988*b*). The only established role for the channels responsible for Ca^{2+} plateaux is to mediate serotonin-induced bistability (Hounsgaard & Kiehn, 1989; Kiehn, 1991*a*,*b*).

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