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NON-SYNAPTIC DEPOLARIZING POTENTIALS IN RAT SUPRAOPTIC NEURONES RECORDED *IN VITRO*

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SUMMARY

1. Intracellular recordings obtained from eighty-two supraoptic nucleus neurones in perfused explants of rat hypothalamus revealed a mean resting membrane potential of -66 ± 5 mV (S.D.) and spike amplitudes of 70–106 mV.

2. When recorded with K acetate-filled micropipettes, non-spike membrane voltage fluctuations included spontaneous depolarizing and hyperpolarizing potentials.

3. Spontaneous hyperpolarizing potentials peaked in 3–5 ms and decayed exponentially with a mean time constant of $20{\cdot}2 \pm 0{\cdot}1$ ms, 1·6 times the membrane time constant of $13{\cdot}8 \pm 0{\cdot}1$ ms. These potentials were identified as spontaneous inhibitory post-synaptic potentials, and all demonstrated a common reversal potential near -80 mV, a depolarizing shift of this reversal potential during intracellular Cl^- accumulation, and reversible blockade by raising $[Mg^{2+}]$ to 15 mM in the perfusate.

4. Depolarizing potentials with features typical of spontaneous excitatory post-synaptic potentials i.e. brief (8–20 ms) depolarizing transients, were rarely recorded with K acetate-filled micropipettes. Instead, most neurones demonstrated what are termed non-synaptic depolarizing potentials (n.s.d.p.s) lasting 20–125 ms (mean $86{\cdot}4 \pm 8{\cdot}6$ ms (S.E. of mean)) with a rise time $21{\cdot}1 \pm 2{\cdot}8$ ms and a decay time of $16{\cdot}3 \pm 2{\cdot}8$ ms ($n = 28$ measured). Unlike typical spontaneous post-synaptic potentials, these events could sustain a constant peak amplitude for most of their duration.

5. These n.s.d.p.s displayed a strong voltage-dependent behaviour and were detected only at membrane potentials within 5–7 mV of the threshold for spike initiation. Spontaneous slow depolarizing membrane shifts preceding or following phasic bursts, or any manipulation (e.g. current step, sinusoid, depolarizing after-potential) causing the membrane potential to enter this range of activation, prompted their appearance.

6. N.s.d.p.s were completely insensitive to the presence of 15 mM-Mg^{2+} but they were reduced in size and frequency when Ca^{2+} were replaced with Co^{2+} or Mn^{2+}. They were detected at a more positive membrane potential when Na^+-dependent action potentials were blocked with tetrodotoxin.

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7. The size, voltage-dependent and non-synaptic nature of these depolarizing potentials raises the possibility that they reflect the activity of individual (or small clusters of) ionic channels carrying inward current. Their ability to serve as prepotentials to trigger spikes is deemed to be particularly important for promoting the onset of phasic bursts in supraoptic neurosecretory neurones.

INTRODUCTION

Intracellular recordings from magnocellular supraoptic nucleus (s.o.n.) and paraventricular nucleus (p.v.n.) neurosecretory neurones *in vitro* indicate that the phasic bursting activity patterns which some of these cells display is generated intrinsically (Andrew & Dudek, 1983, 1984). Each phasic burst is driven by a 4–10 mV 'plateau' potential which arises through the summation of depolarizing after-potentials (d.a.p.s) that follow individual action potentials. However, bursts initiated through this process depend on the initial presence of a sequence of action potentials that are sufficiently rapid in occurrence to permit their d.a.p.s to summate. While a slow depolarization precedes successive bursts in some cells, burst onset in other phasic bursters clearly depends on other mechanisms for spike initiation.

Spontaneous depolarizing voltage fluctuations are frequently recorded in s.o.n. and p.v.n. neurones (e.g. Dudek, Hatton, & MacVicar, 1980; Mason, 1983). When sufficiently large, these potentials can directly trigger action potentials and help to initiate the formation of a burst (Andrew & Dudek, 1984). Many of these fluctuations in membrane voltage may indeed reflect spontaneous synaptic activity. However, we now report on a subpopulation of depolarizing potentials that can be distinguished from typical post-synaptic potentials by their shape, voltage-dependent behaviour and insensitivity to synaptic blockade. The range of activation of these depolarizations is consistent with a role in initiating action potentials and thereby promoting burst onset. A brief communication has been presented (Bourque & Renaud, 1984).

METHODS

Basal hypothalamic explants (8 × 2 × 2 mm) were isolated from male Sprague–Dawley rats (150–300 g) and pinned to the Sylgard (Corning Ltd.) base of a perfusion chamber. The right internal carotid artery was cannulated as previously described (Bourque & Renaud, 1983) and perfused with artificial cerebrospinal fluid (ACSF) at 33 °C. The oxygenated (95% O_2, 5% CO_2) ACSF was delivered by gravity at a rate of 1·0–1·5 ml/min and contained (mM): NaCl, 126; KCl, 3; KH_2PO_4, 1·3; $MgCl_2$, 1·3; $CaCl_2$, 2; $NaHCO_3$, 25·9 and glucose, 10. For purposes of blocking synaptic transmission, the concentration of $MgCl_2$ was raised to 15 mM and that of NaCl lowered to 110 mM. To block or reduce somatodendritic Ca^{2+} currents, $CaCl_2$ was isomolarly replaced with either $MnCl_2$ or $CoCl_2$. Blockade of Na^+-dependent action potentials was achieved by adding 0·2–1·0 μM-tetrodotoxin (TTX, Sigma Ltd.) to the ACSF).

To evoke synaptic responses, a concentric bipolar (0·4 mm o.d.) electrode was positioned into the anteroventral third ventricular (AV3V) area, mid line and immediately rostral to the optic chiasm. Electrical pulses (10–100 μA, 0·05 ms) were delivered by isolated stimulation units (DS-2; Devices Ltd.) controlled by a programmable clock (Digitimer Ltd.).

Intracellular recordings were achieved through micropipettes (1·2 mm o.d., 0·6 mm i.d.) pulled on a Brown-Flaming puller (Sutter Instruments Ltd.) and filled (as specified in the text) with K acetate (3 M) or KCl (3 M). The best impalements were obtained with high-impedence electrodes (150–300 MΩ) having a rise time to peak of less than 0·2 ms during 0·1–0·3 nA current pulses. The electrolyte filling the pipette was connected via a Ag–AgCl electrode to the input stage of a Mentor

N-950 pre-amplifier. The signal was low-pass filtered at 5 kHz and recorded on tape using an FM recorder (Racal Ltd.). Play-back of taped signals at lower speeds could produce chart records having a frequency response of d.c.–1 kHz. Alternatively, photographs were taken from a storage oscilloscope either during or after an experiment. Potential rise times and decay times (from 10 to 90 % of peak) and durations (at one-third amplitude) were measured on a digital oscilloscope.

Impalements were obtained by advancing the electrode (Burleigh Ltd.) in 4 μm steps and applying 20–40 ms depolarizing current pulses (1–5 nA). Recordings obtained within 200 μm of the surface displayed an activity-dependent enhancement of the duration of their action potentials and were therefore presumed to be magnocellular neurosecretory neurones (Bourque & Renaud, 1985*b*). The criteria for an impalement to be considered of adequate quality and subsequent analysis were: (i) a minimum spike amplitude of 70 mV (threshold to peak) at resting potential, (ii) an input resistance greater than 75 MΩ and (iii) sustained repetitive firing during 200 ms depolarizing (0·1–0·2 nA) current pulses.

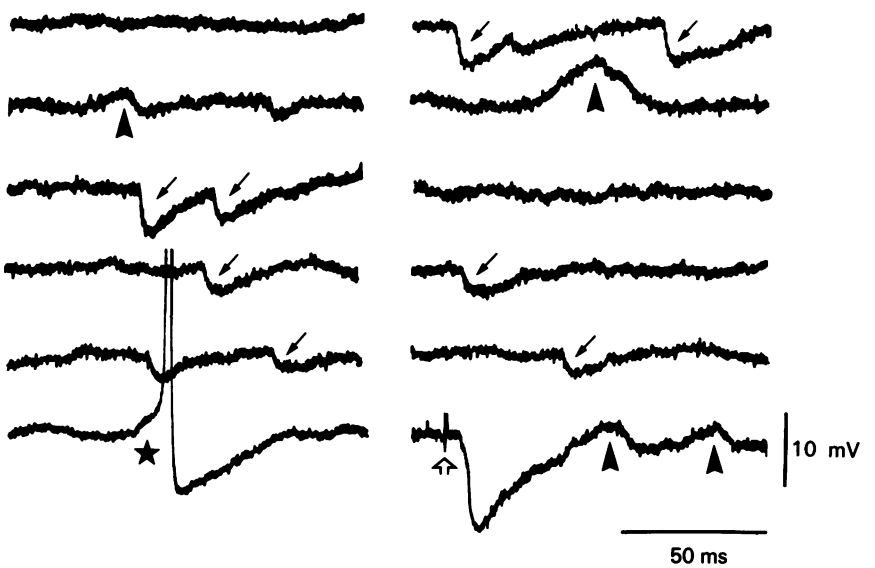

Fig. 1. Example of spontaneous membrane voltage fluctuations recorded from a supraoptic neurone impaled with a K acetate-filled micropipette. At the resting membrane potential (−60 mV), this cell displayed abundant transient hyperpolarizations (slanted arrows) similar in shape to the inhibitory post-synaptic potential that followed electrical stimulation in the anteroventral third ventricular (AV3V) area (shock artifact identified by the open arrow). Several depolarizing potentials (arrowheads) are also present; one of these (★) initiates an action potential.

RESULTS

Intracellular recordings from eighty-two s.o.n. neurones revealed a mean resting membrane potential of -66 ± 5 mV (± S.D.) and spike amplitudes ranging between 70 and 106 mV. At resting potential, sixty-four cells impaled with K acetate filled-electrodes displayed spontaneous depolarizing and hyperpolarizing potentials (Fig. 1). Their nature and ionic basis were further examined by a combination of current injection, intracellular Cl^- ionophoresis and exposure to ACSF containing TTX, Co^{2+}, Mn^{2+}, or Mg^{2+}.

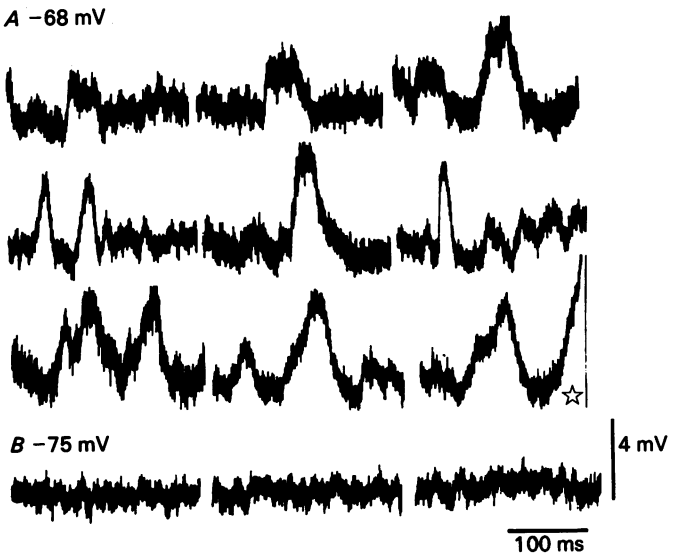

Fig. 2. *A*, nine single oscilloscope sweeps illustrate the variability in size and shape of depolarizing potentials (n.s.d.p.s; see text) recorded from an s.o.n. neurone impaled with a K acetate-filled electrode. In the last sweep, a spike (86 mV) originates from one of these potentials (★). *B*, three representative traces obtained from the same cell held at a membrane potential of −75 mV by current injection no longer reveal depolarizing potentials.

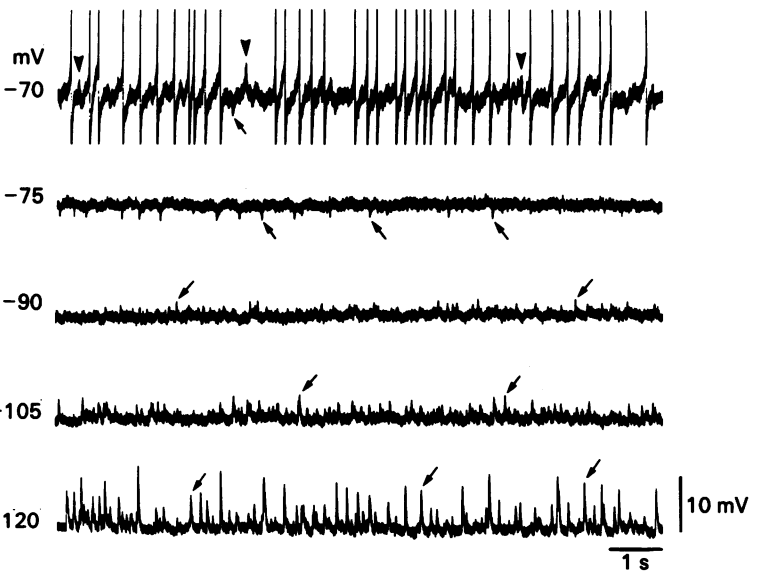

Fig. 3. Chart recordings of an s.o.n. neurone impaled with a K acetate-filled micropipette (spike amplitude was 93 mV). Spontaneous hyperpolarizing potentials (i.p.s.p.s; several are indicated by the slanted arrows) are visible at −70 and −75 mV. Their polarity is reversed at more hyperpolarized potentials. At a membrane potential of −70 mV, occasional isolated depolarizing potentials referred to as n.s.d.p.s (arrowheads) are present; others appear to initiate action potentials and are obscured. Note the absence of n.s.d.p.s at membrane potentials of −75 and −90 mV.

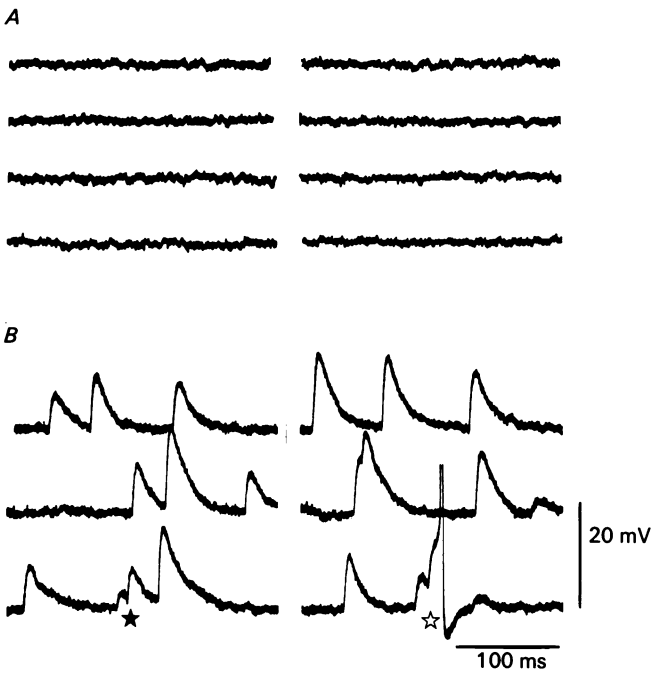

Fig. 4. Oscilloscope recordings obtained from a single s.o.n. neurone impaled with a KCl-filled micropipette. *A*, during the first minute of impalement few voltage fluctuations are apparent. *B*, 7 min after penetration of the cell, numerous spontaneous depolarizing events (reversed i.p.s.p.s) are present; occasionally these summate (★) and trigger an action potential (☆). Note the similar (but inverted) shape of these potentials to the spontaneous hyperpolarizations shown in Fig. 1. All traces were obtained at −75 mV. Spike amplitude (last trace) was 101 mV.

Spontaneous depolarizing potentials

In this preparation, s.o.n. neurones impaled with K acetate-filled electrodes displayed two types of spontaneous depolarizing potentials. Rarely observed were brief events (i.e. 8–20 ms) with a rapid rise to peak and a delayed return to base line similar in configuration to spontaneous excitatory post-synaptic potentials (e.p.s.p.s) recorded in other central neurones. Closer inspection (Fig. 2) revealed that the majority of cells demonstrated 2–10 mV depolarizing potentials that were 20–125 ms in duration (mean 86·4 ± 8·6 ms (S.E. of mean)). Their shapes differed from typical e.p.s.p.s in that they were more symmetric (Fig. 2*A*) with a rise time of 21·1 ± 2·8 ms and a decay of 16·3 ± 2·8 ms ($n = 28$). Several additional features serve to distinguish these potentials from spontaneous synaptic potentials; they are therefore referred to below as non-synaptic depolarizing potentials (n.s.d.p.s).

Spontaneous hyperpolarizing potentials

As illustrated in Fig. 1, intracellular recordings using *K acetate-filled* micropipettes revealed frequent spontaneous hyperpolarizing potentials typical of inhibitory post-synaptic potentials (i.p.s.p.s). These i.p.s.p.s peaked in 3–5 ms and decayed exponentially with a mean time constant of 20·2 ± 0·1 ms. This value is 1·6-fold

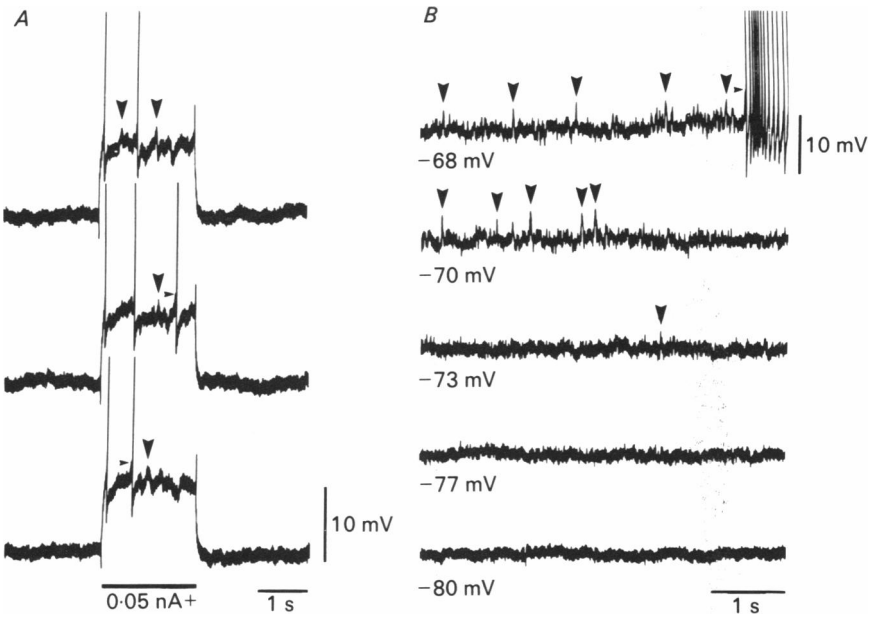

Fig. 5. Voltage dependence of n.s.d.p.s in s.o.n. neurones. *A*, three sweeps obtained with a K acetate-filled electrode illustrate the appearance of n.s.d.p.s (arrowheads) during depolarizing steps (bar) to a membrane potential near the threshold for spike initiation. Some n.s.d.p.s actually trigger spikes (horizontal arrowheads). Note the absence of any depolarizing events at the holding potential of −78 mV, well below spike threshold. *B*, disappearance of n.s.d.p.s during progressive membrane hyperpolarization. In the top trace, an n.s.d.p. triggers a spike (horizontal arrowhead) whose subsequent depolarizing after-potential induces repetitive firing. Note the narrow range of membrane potential within which n.s.d.p.s are observed. Spike amplitude was 81 mV.

greater than the membrane time constant of 13·8 ± 0·1 ms for the decay of hyperpolarizing pulses measured in the same neurones ($n = 9$ cells analysed). In all cells these spontaneous i.p.s.p.s were reduced in amplitude by membrane hyperpolarization and eventually reversed in polarity at membrane potential values more negative than −80 mV (Fig. 3).

Following impalement of s.o.n. neurones with *KCl-filled* micropipettes ($n = 8$ cells tested), few spontaneous potential deflexions of either polarity were seen at resting membrane potentials. However, spontaneous i.p.s.p.s. of reversed polarity and amplitudes sufficient to trigger action potentials appeared within 3–5 min (Fig. 4), reflecting the altered transmembrane Cl^- concentration gradient consequent to intracellular Cl^- diffusion or ionophoresis.

Voltage dependence of n.s.d.p.s

The amplitude and sign of e.p.s.p.s and i.p.s.p.s depend on the algebraic difference between the prevailing membrane potential and the cell's apparent equilibrium potential for Na^+ or Cl^- respectively. If one excludes the depolarizing potentials that are obvious reversed i.p.s.p.s recorded with electrodes containing KCl (Fig. 7*A*) or under conditions of membrane hyperpolarization (Fig. 3), the depolarizations referred to as n.s.d.p.s demonstrate a voltage dependence that is clearly different from that

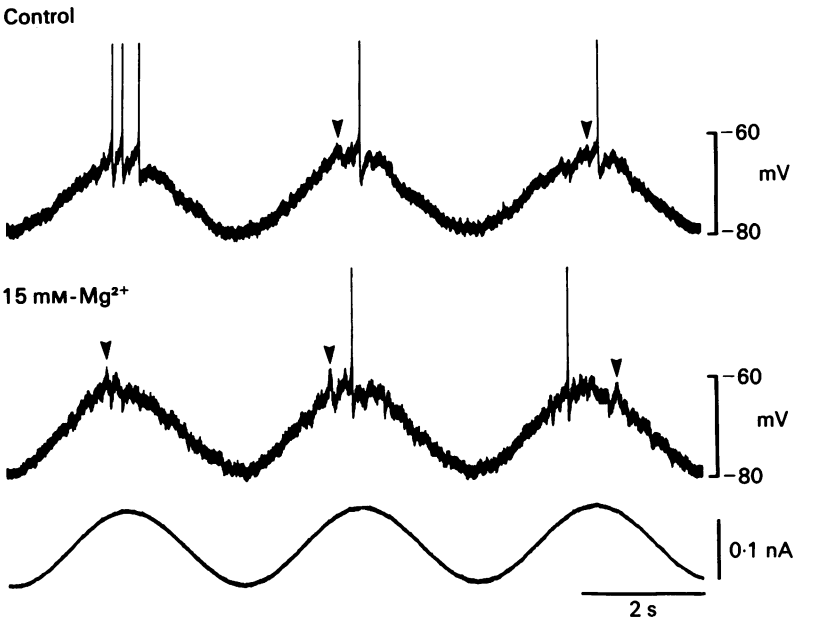

Fig. 6. The upper voltage traces following injection of a sinusoid current wave (lowest trace) illustrate the voltage-dependent generation of n.s.d.p.s (arrowheads) in an s.o.n. neurone impaled with a K acetate-filled electrode; n.s.d.p.s appear only during the depolarizing phase in both normal media and ACSF containing 15 mM-Mg^{2+}. The lower trace was obtained after 20 min of perfusion in high Mg^{2+} (14 min after blockade of the AV3V-evoked i.p.s.p; cf. Fig. 3 in Bourque *et al.* 1985). Spike amplitude was 76 mV.

expected of Na^+-dependent e.p.s.p.s. Thus, n.s.d.p.s are observed only at membrane potentials within 5–7 mV of the threshold for spike initiation. In all cells, membrane hyperpolarization beyond *ca.* −70 mV was invariably associated with their disappearance (Figs. 2 and 5). Their presence could then be revealed by stepwise removal of hyperpolarizing current in small increments from a holding potential below −75 mV until rapid firing of action potentials obscured their presence (Fig. 5*B*). Their appearance at or near −70 mV could be revealed by any form (i.e. step, ramp or sinusoid) of current injection causing the membrane potential to enter and/or leave their narrow range of activation (Figs. 5*A* and 6). In addition to direct current injection, the appearance of n.s.d.p.s could be induced during the prolonged d.a.p.s that follow short (50–200 ms) current-evoked bursts of action potentials (Fig. 7). Regardless of the method used to induce their appearance in a given cell, the estimated threshold potential was the same.

Sensitivity to Mg^{2+}

The strong voltage dependence of the appearance of n.s.d.p.s suggests that these events do not represent a conventional form of chemically mediated synaptic activity. This apparent intrinsic or non-synaptic origin was further investigated in seventeen cells exposed to 15 mM-Mg^{2+}, sufficient to block both synaptically evoked i.p.s.p.s (Fig. 3 in Bourque, Randle & Renaud, 1985) and spontaneously occurring i.p.s.p.s (Fig. 7*A*) in this preparation. In the presence of high Mg^{2+}, n.s.d.p.s

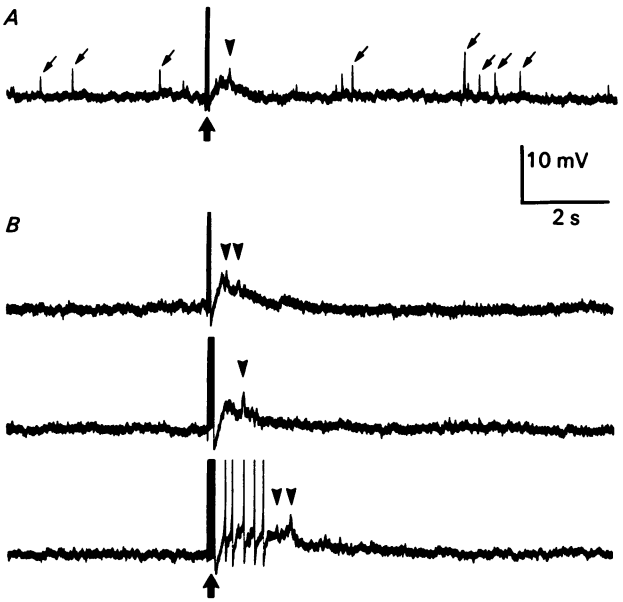

Fig. 7. Synaptic and non-synaptic depolarizing responses recorded from an s.o.n. neurone impaled with a KCl-filled electrode. *A*, this record shows several spontaneous depolarizing potentials (slanted arrows) which, at a holding potential of −71 mV have the configuration of reversed i.p.s.p.s (cf. Figs. 3 and 4). One n.s.d.p. (arrowhead) is also seen during a depolarizing after-potential that follows the 50 ms burst of four current-evoked (0·2 nA) action potentials (arrow). *B*, three consecutive traces obtained from the same cell after 20 min of perfusion with ACSF containing 15 mM-Mg^{2+}. Note the absence of inverted spontaneous i.p.s.p.s (holding potential −71 mV) but the continued presence of n.s.d.p.s during the depolarization provided by the current-evoked depolarizing after-potentials.

continued to appear either as a result of direct current injection (Fig. 6) or during the d.a.p.s following brief current-evoked bursts of spikes (Fig. 7*B*).

Effects of TTX

The addition of 0·2–1·0 μM-TTX to the ACSF removes the Na^+ component of the action potential and reveals a high-threshold Ca^{2+} spike in s.o.n. neurones (Bourque & Renaud, 1985*a*). In the presence of TTX, n.s.d.p.-like potentials continued to appear in eleven out of fourteen cells tested (Fig. 9) and could now be elicited by depolarizations beyond −55 mV, i.e. within *ca.* 10 mV of the threshold for Ca^{2+} spike initiation.

Effects of Co^{2+} and Mn^{2+}

In s.o.n. neurones elevations of Mg^{2+} concentrations in the ACSF do not appear to block somatodendritic influx of Ca^{2+} since d.a.p.s, after-hyperpolarizations and spike broadening are unaffected (C. W. Bourque & L. P. Renaud, unpublished observations). However, Co^{2+} and Mn^{2+} do block Ca^{2+} spikes in s.o.n. neurones (Bourque & Renaud, 1985*a*). In six out of six cells tested, replacement of Ca^{2+} by Co^{2+} or Mn^{2+} in ACSF containing TTX eliminated the appearance of both n.s.d.p.s and Ca^{2+} spikes.

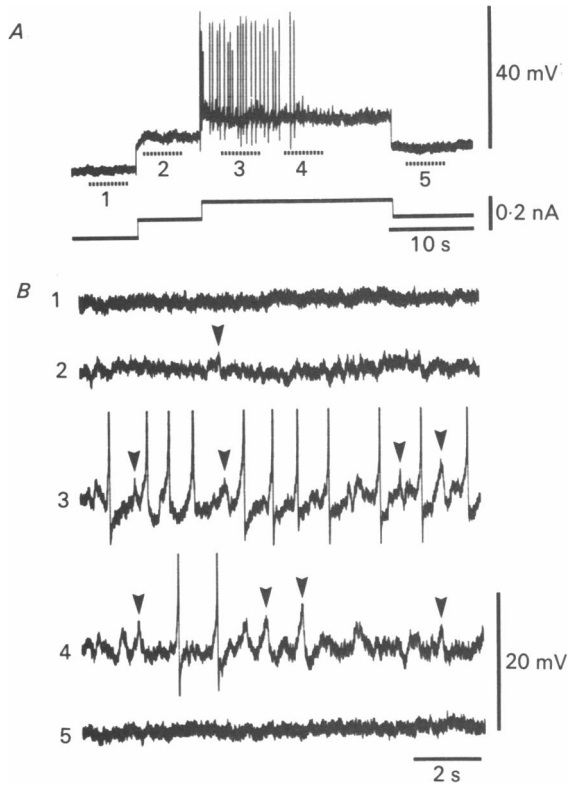

Fig. 8. Generation of n.s.d.p.-like potentials (arrowheads) in the presence of TTX. *A*, chart record of a s.o.n. neurone held at different membrane potentials by current injection through the electrode. *B*, expanded versions of the records underlined in *A*. Actual membrane potentials were: 1, −60 mV; 2, −53 mV; 3 and 4, −49 mV; 5, −57 mV.

In ACSF containing no TTX, Co^{2+} or Mn^{2+} reversibly reduced but did not abolish their appearance during membrane depolarizations near the threshold for spike initiation (Fig. 9).

N.s.d.p.s appear during phasic bursting

Data from the present studies, in agreement with observations derived from hypothalamic slice preparations (Hatton, 1982; Andrew & Dudek, 1983, 1984), imply that phasic firing does not result from patterned synaptic input. When phasic firing was abolished by membrane hyperpolarization in sixteen cells, examination of high-gain records failed to reveal the occurrence of patterned synaptic depolarizations. In these same cells recorded during spontaneously occurring bursts, the slow membrane depolarizations that led up to, or followed a phasic burst were accompanied by depolarizing events identical to n.s.d.p.s (Fig. 10). Most of the phasic bursts recorded in our experiments appeared to be initiated by summation of d.a.p.s. following spikes triggered by n.s.d.p.-like depolarizations (e.g. Fig. 5*B*). These n.s.d.p.s. could be eliminated by hyperpolarizing the membrane to potentials below *ca.* −70 mV, and they reappeared upon removal of the hyperpolarizing current.

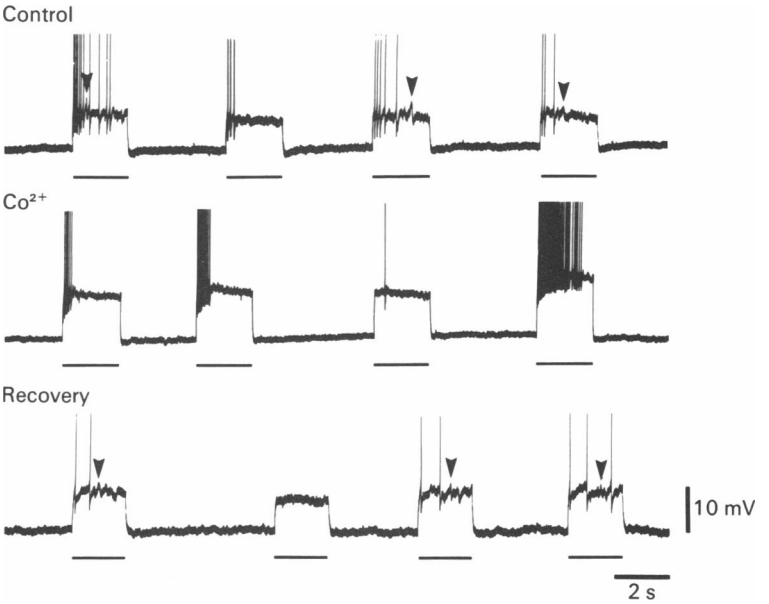

Fig. 9. Effects of Co^{2+} (3 mM) on the generation of n.s.d.p.s during membrane depolarization evoked by current injection (bars). Note the reversible reduction in the number and size of n.s.d.p.s (arrowheads) at membrane potentials near the threshold for spike initiation (spike amplitude 93 mV) when Ca^{2+} is replaced by Co^{2+}. K acetate-filled micropipette.

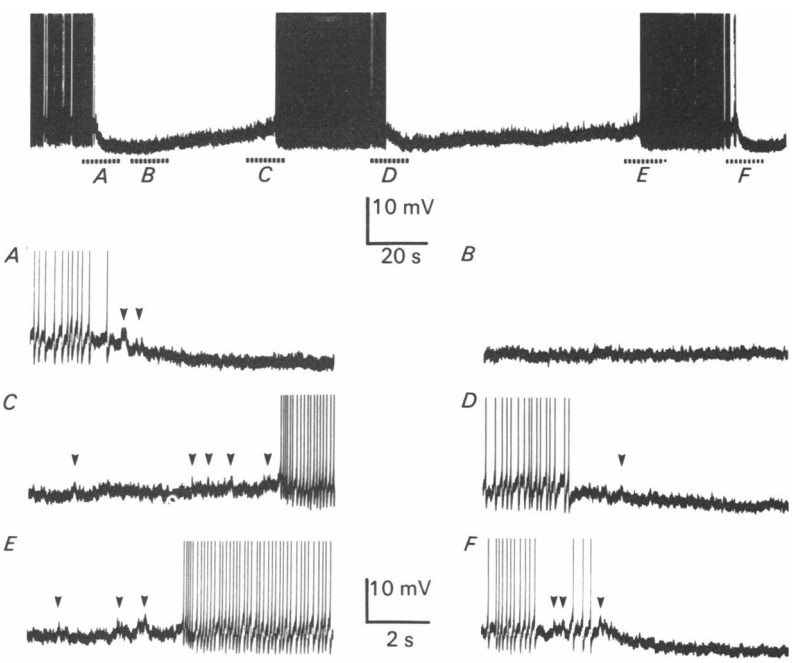

Fig. 10. Phasic firing in a s.o.n. neurone recorded with a K acetate-filled micropipette. *A–F* are expanded segments of the top trace. Spike amplitude was 96 mV. In this cell, bursts were preceded by a slow depolarizing wave. Note the appearance of n.s.d.p.s (arrowheads) immediately before (*C* and *E*) and near the end of (*A*, *D* and *F*) but not between each burst. The peak of the interburst hyperpolarization (between *A* and *B*) was −75 mV.

During the spontaneous burst cycle, n.s.d.p.s disappeared during the interburst hyperpolarization if the corresponding membrane potential dropped below −70 mV.

DISCUSSION

Our intracellular recordings in s.o.n. neurones reveal the presence of spontaneous depolarizing events that are uniquely different from spontaneous post-synaptic potentials by their shape and duration, their voltage dependence, their presence in TTX solutions and elevated concentrations of Mg^{2+}, and their sensitivity to Co^{2+} and Mn^{2+}. Although the mechanism underlying the generation of these n.s.d.p.s cannot be determined from the present experiments, three possibilities may be suggested.

First, the existence of dye-coupling among s.o.n. neurones suggests the possible existence of electrotonic synapses or coupling (Andrew, MacVicar, Dudek & Hatton, 1981). However, the shape of n.s.d.p.s is inconsistent with the low-pass filtering characteristics of electrical junctions (Getting, 1974).

Secondly, n.s.d.p.s could represent decremented remnants of depolarizing events occurring at remote sites (e.g. in the dendrites) and conducted electrotonically through a non-active zone (Spencer & Kandel, 1961; Llinas & Nicholson, 1971). However, the wide variety of shapes and durations of n.s.d.p.s recorded from individual s.o.n. neurones would require many 'trigger' zones (e.g. dendritic bifurcations) to be located at sites electrotonically distant from the recording point (presumably the soma). This interpretation appears unlikely since recent anatomical reconstructions indicate that the dendritic arbour of s.o.n. cells comprises few principal (two to ten) bifurcations (Randle, Bourque & Renaud, 1986), most of which are well within one length constant of the soma (Bourque, 1984).

Finally, the activity of individual or small clusters of ionic channels carrying an inward current of the order of 10 pA could account for depolarizations of a few millivolts given the high input resistance (75–400 MΩ) of s.o.n. neurones. Intrasomatic recordings of membrane-voltage fluctuations corresponding to single-channel activity have previously been reported for other types of secretory cells (e.g. Atwater, Dawson, Eddlestone & Rojas, 1981; Fenwick, Marty & Neher, 1982; Maruyama & Petersen, 1982; Mason & Waring, 1985).

While the effects of Ca^{2+} channel blockers appear to indicate a sensitivity of the mechanism underlying n.s.d.p. generation to these agents, n.s.d.p.s need not necessarily reflect a Ca^{2+}-dependent process. In the presence of TTX, n.s.d.p.-like events can be recorded but their threshold is shifted in the depolarizing direction. Either with or without TTX, n.s.d.p.s begin to appear when the membrane potential is within *ca.* 10 mV of the threshold for the respective spike (i.e. Na^{2+} or Ca^{2+}). In other words, n.s.d.p.s may represent a form of local response which is clearly resolved because of the large input resistance of s.o.n. cells. The effects of Mn^{2+} and Co^{2+} could simply result from a shift in the activation curve of these channels due to surface charge effects.

Physiological roles of n.s.d.p.s

The exact ionic mechanism underlying n.s.d.p. generation clearly warrants further investigation since their ability to trigger action potentials in rapid succession

facilitates and maximizes the summation of d.a.p.s and the formation of the plateau depolarization which underlies each burst. During membrane depolarizations associated with increases in osmotic pressure (Mason, 1980; Abe & Ogata, 1982) or exposure to certain neurotransmitters, e.g. noradrenaline (Randle, Day, Jhamandas, Bourque & Renaud, 1986), the induction of n.s.d.p.s may help initiate firing. In sufficiently depolarized cells, the continuous presence of n.s.d.p.s may lead to bursting activity by repeatedly triggering bursts as the cell recovers from the refractory mechanisms which terminate firing and produce the interburst interval. In cells whose bursts are preceded by a slow depolarizing wave, the induction of n.s.d.p.s above *ca.* −70 mV is a crucial part of burst onset.

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